Environmental Assessment
of
AQUI-S® E10% (Eugenol Immersion Solution)
For Field-Use in
Freshwater And Saltwater
Finfish

AQUI-S New Zealand, LTD

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1 Introduction

In accordance with the Code of Federal Regulations [21 CFR 25.15(a)], all applications or petitions requesting agency action require the submission of an environmental assessment (EA) or a claim of categorical exclusion (CE) to evaluate whether the proposed action (i.e., the approval of an original new animal drug application (NADA)) may have a significant impact on the human environment. This document, therefore, provides an assessment of potential environmental impacts posed by the original NADA approval of AQUI-S® E10% (eugenol immersion solution), for field-use in freshwater finfish (i.e., salmonids and non-salmonids) to achieve a sedation to handleable condition.[1] Field-use covers sedation of wild populations of finfish for resource management for spawning, tagging, weighing and measuring, and other activities and does not cover uses for fish held in confined settings such as hatcheries, ponds, and aquariums. In addition, this document includes an assessment of potential environmental impacts

[1] Finfish are expected to be sedated to a handleable condition within minutes (mean approximately 1 – 3 minutes) when exposed to the low end of the dose range. The finfish may be released back into the natural waterbody immediately after recovery from sedation, which takes on average 4 – 12 minutes when exposed to the low end of the dose range.

associated with a future supplemental NADA covering field-use in saltwater finfish (i.e., salmonids and non-salmonids).

Three direct pathways of introduction of eugenol into the environment are evaluated herein: (1) streamside, lakeside and shoreside[2] use, (2) use on freshwater electroshocking boats, and (3) use on marine boats. In addition, the potential for secondary poisoning of non-target scavenger or predator species that may consume wild fish treated with eugenol was evaluated as an indirect pathway of introduction.

The assessment follows the Center for Veterinary Medicine (CVM) Phase I and Phase II Environmental Impact Assessment guidance documents [Guidance for Industry (GFI) 89 and 166, respectively].[1, 2] The assessment consists of (1) a summary of the proposed use of AQUI-S® E10%; (2) a description of the chemical characteristics and physico-chemical properties of eugenol and relevant excipients; (3) predicted environmental concentrations of eugenol in surface water (PEC_{SW}) from the three direct pathways of introduction of eugenol into the environment and the one indirect introduction pathway related to secondary poisoning; (4) estimates of the adverse effects concentration(s) of eugenol to non-target species; and (5) a risk characterization for the proposed use of AQUI-S® E10%.

2 Purpose and need

An original NADA approval is being sought for the use of AQUI-S® E10% (eugenol immersion solution) for sedation to a handleable condition in freshwater finfish (field-use only) at the following dose ranges: 25 – 40 mg L^{-1} eugenol for freshwater salmonids, and 40 – 100 mg L^{-1} eugenol for freshwater non-salmonids (see Table 2.1). In addition, it is anticipated that, in the future, a supplemental NADA will be sought for the use of AQUI-S® E10% for sedation to a handleable condition in saltwater finfish (field-use only) at the following dose ranges: 25 – 40 mg L^{-1} eugenol for saltwater salmonids, and 30 – 40 mg L^{-1} eugenol for saltwater non-salmonids (see table 2.1). This animal drug is needed for resource management purposes to allow for fish to be sedated for handling for spawning, tagging, weighing and measuring, and other activities. This proposed use does not cover fish held in confined settings such as hatcheries, ponds, and aquariums.

Table 2.1. Proposed doses.

Finfish Species	Eugenol Concentration [mg L^{-1}]
Freshwater Salmonids	25 – 40
Freshwater Non-salmonids	40 – 100
Saltwater Salmonids	25 – 40
Saltwater Non-salmonids	30 – 40

[2] Shoreside use is defined as use of AQUI-S® E10% next to estuarine and marine waterbodies (e.g., ocean, bay, tidal rivers).

3 Scope of the assessment

The assessment contained herein covers both the current proposed field-use in freshwater finfish and the anticipated future proposed field use in saltwater finfish.

The proposed doses in Section 2 guided the development and evaluation of three exposure scenarios that encompass the range of proposed doses for salmonids and non-salmonids (both freshwater and saltwater), in multiple anticipated use settings capturing the direct pathways of introduction for eugenol: (1) streamside, lakeside and shoreside[2] use, (2) use on freshwater electroshocking boats, and (3) use on marine boats (See Sections 6.1 – 6.4). These exposure scenarios are intended to conservatively characterize expected use patterns based on the proposed conditions of use and anticipated use settings. The specific exposure scenarios evaluated in the EA are detailed in Table 3.1 below.

Table 3.1. Use patterns, tank volumes, and eugenol concentrations evaluated in this assessment.

Exposure Scenario	Evaluated Dosage Range and Tank Volume
Streamside, lakeside, and shoreside	25 – 100 mg L^{-1} eugenol in 5 – 100-gal vessel
Electroshocking boats	25 – 100 mg L^{-1} eugenol in 50 – 100-gal vessel
Marine boats	15 – 40 mg L^{-1} eugenol in 50 – 250-gal vessel

In all cases, fish are immersed in the AQUI-S® E10% solution until sedation to a handleable condition is achieved, processed, and then released. After the fish are treated, the AQUI-S® E10% solution in the tanks is disposed to surface water. After disposal, non-target organisms (e.g., invertebrates, fish) may be exposed to residues of eugenol. Due to this potential for exposure, the environmental fate and effects of eugenol in the aquatic environment were assessed, and a risk characterization of this exposure pathway was conducted.

In addition, the potential impacts to scavenger or predator species (i.e., carnivorous birds, fish, and mammals) that may consume wild fish or wild fish carcasses[3] containing residues of eugenol following sedation and handling at sites of use were assessed (see Section 7.5).

4 Identification of substances subject to the proposed action

AQUI-S® E10% is a 10% eugenol weight per weight (w/w) solution containing 112 mg eugenol per mL intended to be mixed directly with water to produce an anesthetic bath. To aid in dispersal and stability of eugenol, two excipients are included in the AQUI-S® E10%

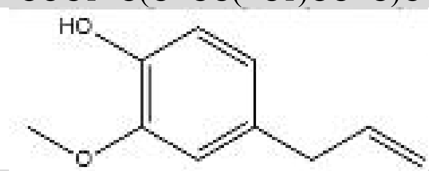
[3] This exposure pathway results from the disposal of treated fish carcasses at the site of use (e.g., streamside, lakeside), which is expected to be a rare and sporadic occurrence.

formulation: PEG 400 (polyethylene glycol; 89% of AQUI-S® E10%) and polysorbate 80 (1% of AQUI-S® E10%). A brief description of the components of AQUI-S® E10% follows.

4.1 Eugenol

Eugenol is an allyl chain-substituted guaiacol and is a member of the allylbenzene class of chemical compounds. It is a clear to pale yellow oily liquid extracted from certain essential oils, especially from clove oil.[3] Select chemical characteristics are presented in Table 4.1.

Table 4.1. Select chemical characteristics for eugenol[4]

Characteristic	Value
Systemic chemical name	2-methoxy-4-(2-propenyl)phenol
Chemical Abstracts Service (CAS) number	97-53-0
Molecular formula	$C_{10}H_{12}O_2$
Molecular weight	164.2 g mol^{-1}
Canonical SMILES string	COC1=C(C=CC(=C1)CC=C)O
Chemical structure	
Mode of action	Eugenol is an anesthetic that causes immobility in fish by reducing the respiratory rate and depressing the central nervous system (e.g., reduces heart rate, cardiac output, dorsal aortic pressure, and arterial oxygen).[5]

4.2 Excipients

AQUI-S® E10% is comprised of 89% PEG 400 and 1% polysorbate 80. These excipients are discussed in the following sections.

4.2.1 Polyethylene glycol (PEG) 400

Polyethylene glycols, also known as PEGs, are clear, colorless, thick liquids to waxy solids, depending on the molecular weight. The molecular weight of PEGs ranges from 200 to over 6000 g mol^{-1}. Some may have a faint odor and bitter taste. The formula of PEG is expressed as $H(OCH_2CH_2)_nOH$. The number included in the name of PEG 400 indicates the average molecular weight (e.g., a PEG where n = 9 would have an average molecular weight of approximately 400 g mol^{-1} and be labeled PEG 400).[6] Select chemical characteristics are presented in Table 4.2.

Table 4.2. Select chemical characteristics for PEG 400.[6]

Property	Value
CAS Number	25322-68-3
Molecular Formula	$HO(C_2H_4O)_n H$
Molecular Weight	380 – 420 g mol^{-1}
Aqueous Solubility, pH 7[7]	100% at 20 °C mg L^{-1}
Density	1.13 g cm^{-3} (20 °C)
pH	5 – 7 (100 g L^{-1}, H_2O, 20 °C)

Because 89% of the AQUI-S® E10% formulation is comprised of PEG 400, an analysis of the fate and effects of this excipient was conducted.[8] A summary of that evaluation is included below.

PEG 400 is practically involatile and is hydrolytically stable. Under aerobic conditions, polyethylene glycol is degraded by dehydrogenation to either carboxylated intermediates or glycoaldehyde, by acetoaldehyde production, and by extracellular hydrolytic cleavage with production of ethylene glycol (EG) and diethylene glycol.[9] It is readily biodegradable with 90% degradation observed in 28 days following Organization for Economic Cooperation and Development (OECD) Guideline 301, Method F.[10] Conservative predicted no effect concentration (values for PEG 400 were derived for two algal species, an invertebrate (*Daphnia magna*), and five fish species. In all cases, the predicted no effect concentrations (PNEC) were > 10 mg L^{-1} and the most sensitive species was algae. Given that algae are ubiquitous within lotic and lentic ecosystems and demonstrate rapid recovery, there is a very low likelihood of population-level effects resulting from exposure to the PEG 400.

4.2.2 Polysorbate 80

Polysorbate 80 is a yellow to orange colored, oily liquid with a faint odor and bitter taste.[11] Select chemical characteristics are presented in Table 4.3.

Table 4.3. Select chemical characteristics for polysorbate 80.[11]

Property	Value
CAS Number	9005-65-6
Molecular Formula	$C_{64}H_{124}O_{26}$
Molecular Weight	1,308 g mol^{-1}
Aqueous Solubility, pH 7	~1,900 mg L^{-1} at 25 °C
Density	1.06 – 1.10 g cm^{-3}
pH	6 – 8 (5% solution)

Polysorbate 80 is used as an emulsifier, dispersing agent, surfactant, defoaming agent, synthetic flavoring, stabilizer and thickener in foods, pharmaceuticals, cosmetics and pesticide products. As polysorbate 80 consists of only 1% of the total volume of AQUI-S® E10% and only small volumes of AQUI-S® E10% relative to waterbody sizes will be used at one-time (see Table 2.1), the overall potential exposure to polysorbate 80 in surface waters is considered to be extremely low. Further, Gosselin (1976) reported that polysorbate 80 is “practically nontoxic”[12] for humans. Therefore, a detailed risk assessment is unnecessary due to the very low likelihood of significant effects to the environment.

5 Physico-chemical and fate properties of eugenol

The physico-chemical, adsorption, and degradation properties of eugenol were obtained from studies conducted to support the approval of AQUI-S® E10% and from scientific literature. A description of the data for each property and the property's applicability towards the assessment conducted herein follows.

5.1 Density and solubility

The density of eugenol is reported as 1.064 to 1.070, 1.0652 (68 °F), 1.068 (20 °C) and 1.065 mg L^{-1} (25 °C; the latter two were obtained as part of an OECD Guideline 105 study).[13-16] The average density is 1.066 mg L^{-1}.

Qualitatively, eugenol is described as moderately to slightly soluble.[15] The National Toxicology Program Chemical Repository Database reports the solubility as < 1,000 mg L^{-1} (68 °F).[14] The United States (US) Environmental Protection Agency (EPA) Distributed Structure-Searchable Toxicity (DSSTox) database reports the solubility as 0.03M (= 4,930 mg L^{-1})[17] and the Handbook of Aqueous Solubility Data reports the solubility as 2,460 mg L^{-1} (25 °C).[18] A European Chemicals Agency (ECHA) registration dossier cites an OECD Guideline 105[13] study (flask method) in which the solubility is reported as 1,154 mg L^{-1}.[16] The average of measured solubility values is 2,848 mg L^{-1}. Although the solubility of eugenol is not used quantitatively in this assessment, the data supports that eugenol will dissolve readily in water and solid residues would not be expected to precipitate after disposal of AQUI-S® E10% immersion solutions to surface water because dose concentrations (maximum of 100 mg L^{-1}) are substantially lower than the solubility limit.

5.2 Octanol-water partitioning

The log octanol-water partition coefficient (log K_{ow} or logP) for eugenol is reported by two references as 2.27 L kg^{-1} and 2.49 L kg^{-1}.[17, 19] In the ECHA registration dossier, the log K_{ow} is reported as 1.83 L kg^{-1} from an OECD Guideline 117 study.[16, 20] The geometric average[4] log K_{ow} value is 2.18 L kg^{-1} and is used as the representative log K_{ow} throughout this document.

5.3 Bioconcentration

The geometric average log K_{ow} of 2.18 L kg^{-1} (see Section 5.2) is well below the criterion (log $K_{ow} \geq 4$) for an assessment of bioaccumulation according to CVM's GFI 166.[2] Thus, no assessment of bioaccumulation was conducted herein.

5.4 Sorption

In the environment, eugenol is likely to moderately adsorb to particulate and dissolved organic carbon in the water column and organic matter in sediments. Adsorption can serve to reduce the concentration of eugenol available to water column dwelling biota. To determine the magnitude of sorption, a soil-water adsorption/desorption study was conducted in accordance with OECD

[4] The geometric average of $\log_{10}$ transformed values (e.g., log K_{ow}) is mathematically identical to the arithmetic average of the linear values (e.g., K_{ow}). Reporting partition coefficients, such as the K_{ow}, is commonly done using $\log_{10}$ transformed values.

Guideline 106 (see Appendix B.3).[24, 25] Five soils were tested and linear and Freundlich adsorption isotherms were constructed. Although the study reported equilibrium partition coefficients for eugenol, the study could not complete the mass balance and it appeared that substantial mass loss (e.g., via degradation, sorption to the glass, and/or formation of non-extractable residues) occurred. Thus, the reported equilibrium partition coefficients did not correspond to true equilibrium.

Therefore, to use the data obtained from this study quantitatively, an alternate approach was used. In the alternative approach, the adsorption partition coefficient (K_d) values obtained in biotic and abiotic systems for early sampling points (e.g., 2-hr) were compared to qualify the effect of degradation on the partition coefficient. If biotic and abiotic systems showed similar partitioning behavior and the K_d values were approximately the same, it could reasonably be assumed that the observed K_d values would be due to sorption only. Moreover, as the value of K_d would be expected to increase with time (i.e., more eugenol would sorb to soil as the experiment continued), K_d values for early sampling points would conservatively estimate the water concentration of eugenol, as compared to a K_d value at equilibrium. From the evaluation conducted, it was found that the 2-hr adsorption time data did not appreciably differ between biotic and abiotic systems. Therefore, the sorption coefficients for the 2-hr adsorption period are conservative estimates of the equilibrium partition coefficient. These 2-hr K_{oc} values are shown in Table 5.1

To corroborate that the sorption parameters estimated in the study are reasonable, K_{oc} values were predicted by the US EPA EPI SUITE™ (Estimation Programs Interface (EPI) Suite™) KOCWIN™ v2.0.1 module and the ACD/Labs Percepta Platform – PhysChem module.[26, 27] These models use quantitative structure-activity relationships (QSARs) to predict the K_{oc}. The QSAR models are appropriate, and likely to be relatively accurate, for eugenol due to its small size and well characterized molecular fragments. KOCWIN predicts the K_{oc} as 682 L kg^{-1} (MCI method) while Percepta predicts the K_{oc} as 532 L kg^{-1}.

Table 5.1. Organic carbon (OC) normalized soil-water partition coefficients (K_{oc}) for eugenol.

Soil Name	Soil Texture	% OC	K_{oc} [L kg^{-1}]	
			Non-sterile	Sterile
TB-PF	Silt Loam	4.2	400	357
MSL-PF	Sandy Loam	2.0	645	605
Roger Myron	Loamy Sand	1.2	658	533
DU-Loam	Clay Loam	4.3	605	560
Montana	Clay	0.8	1,050	1,200
Mean K_{oc}			*672*	*651*
Mean log K_{oc}			*2.83*	*2.81*

Thus, the mean 2-hr K_{oc} values from the OECD Guideline 106 study are supported by the EPISUITE and Percepta predicted values. As an additional conservatism in the current assessment, the lower of the mean K_{oc} values obtained for non-sterile and sterile soils is used (i.e., K_{oc} = 651 L kg^{-1}). Use of the lower value will result in a more conservative estimate because it will assume more eugenol is in the water column, which is the compartment of concern for this assessment.

5.5 Degradation

Eugenol has been found to rapidly dissipate[5] in aerobic systems (both soil and water). Public literature and studies conducted to support the approval of AQUI-S® E10% indicate dissipation half-lives on the order of hours and mineralization half-lives on the order of weeks. Although none of the degradation data are used quantitatively in this assessment, the data support that residues of eugenol will rapidly dissipate in the environment shortly after disposal to surface waters. Descriptions of the degradation of eugenol in soil, in water, and via photolysis follows.

5.5.1 In soil

Although aerobic degradation of eugenol in soil may occur and, based on the studies used to support the approval of AQUI-S® E10%, is likely to be rapid, this is not a relevant exposure pathway for the proposed use of AQUI-S® E10% because AQUI-S® E10% is expected to be disposed of directly to waterbodies.

5.5.2 In water

The aerobic mineralization of eugenol in surface waters was evaluated in accordance with OECD Guideline 309 (see Appendix B.2).[28, 29] The objectives of this study were to (1) measure the rate of transformation of eugenol (^{14}C radiolabeled), (2) measure the rate of mineralization of eugenol and/or transformation products, and (3) quantify transformation products and perform a mass balance. Biotic and abiotic systems were used for this evaluation. Although substantial mineralization was observed (approx. 50-70% of applied radioactivity by day 60), a kinetic analysis of data was not conducted, and transformation products were not quantified or identified. Thus, most of the study objectives were not met. However, despite these flaws, the biotic study was well controlled and, due to the degree of mineralization observed, qualitatively supports that eugenol rapidly dissipates from surface waters due to biotic degradation.

The European Chemicals Agency (ECHA) Registration Dossier for eugenol contains information of the properties and tests carried out with eugenol.[16] The Dossier outlines a screening test for the biodegradation of eugenol (3 mg L^{-1}) in water under aerobic conditions. This was carried out as per European Union (EU) Method C.4-E (Determination of the "Ready" Biodegradability – Closed Bottle Test) in compliance with Good Laboratory Practices (GLP). Test water was inoculated with secondary effluent from domestic sewage and aerated for 4 days prior to addition of the eugenol. The biodegradability of eugenol was determined by the degree of dissolved oxygen consumed. On day 7, approximately 50% of the test substance was degraded and by the end of the study (day 28) 82% had been degraded. The conclusion of this study was that "[a]ccording to the OECD-Guidelines the substance has been classified as readily biodegradable."

5.5.3 Via photolysis

No quantitative data regarding the photolysis of eugenol is available in the literature.[30] Eugenol absorbs UV light at wavelengths >290 nm, and, therefore, may be susceptible to direct photolysis.[31] However, because quantitative data do not exist and because the likelihood of

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[5] Dissipation in this context refers to the net effect of multiple fate processes that would all serve to reduce concentrations of eugenol in water (e.g., biotic and abiotic degradation, sorption, hydrolysis, and/or photolysis).

photolysis is reduced in waters due to turbidity, sunlight intensity, and other factors, the effect of photolysis on environmental concentrations of eugenol is not considered in this assessment.

5.5.4 *Summary of degradation*

Eugenol is expected to quickly dissipate in surface waters due to degradation and mineralization, with half-lives ranging from hours to days. Although the available literature qualitatively supports the degradation of eugenol in surface waters, a quantitative evaluation of dissipation via this mechanism was not conducted in this assessment. Degradation would only serve to reduce the exposure concentrations of eugenol in surface waters. No information exists regarding the potential for eugenol to degrade via hydrolysis.[30] Therefore, this environmental fate pathway is not considered in this assessment.

5.6 Summary of the physico-chemical and fate properties of eugenol

From the data available in the literature and from studies conducted to support the approval of AQUI-S® E10%, the physico-chemical and fate properties were determined for eugenol. The values used in this assessment are shown in Table 5.2.

Table 5.2. Physico-chemical and fate properties for eugenol used in this assessment.

Property	Value		Notes
Density	1.066	[mg L^{-1}]	Average (n = 5)
Solubility	2,848	[mg L^{-1}]	Average (n = 3)
Dissociation constant			
Octanol-water partition coefficient (log K_{ow})	2.18	[L kg^{-1}]	Geometric average (n = 3)
Organic carbon soil-water partition coefficient (log K_{oc})	2.81	[L kg^{-1}]	Geometric average (n = 5; f_{oc} = 0.008 – 0.043)
Metabolization			
Mammals	94-100% excreted within 24-hr		
Fish	Approximate excretion half-life of 2-hr		
Degradation			
Water	Rapid[6]		Qualitative data only

6 Ecosystems at the site of introduction

AQUI-S® E10% is proposed for field-use to achieve a sedation to handleable condition in finfish. Field-use covers sedation of wild populations of finfish for resource management for spawning, tagging, weighing and measuring, and other activities, and does not include uses for fish held in confined settings such as hatcheries, ponds, or aquariums. As described in Section 2, the proposed uses for AQUI-S® E10% guided the development of three exposure scenarios that encompass the range of proposed dosages for salmonids and non-salmonids in anticipated use settings (see Table 3.1).

In all cases, fish are immersed into the AQUI-S® E10% solution until sedation to a handleable condition is achieved, removed from the tanks for handling (e.g., fin clipping, health inspection),

[6] The term *rapid* is used as a qualitative estimate of degradation based on the degree of mineralization observed during the aerobic mineralization in surface water study (see Appendix B.2).

and then placed into clean water. After recovery from sedation, fish are released back into the waterbody from which they were obtained. In addition, the immersion tanks containing residues of AQUI-S® E10% are discharged directly to the surface water at the site of use. Therefore, freshwater and saltwater ecosystems were the sites of introduction evaluated in this EA (see Section 7).

7 Exposure assessment

The assessment of the direct exposure scenarios for AQUI-S® E10% was performed by estimating the environmental concentration of eugenol in the water column portion of surface waters. Based on the proposed conditions of use and ecosystems at the site of introduction, three direct exposure scenarios were evaluated and are intended to conservatively characterize the expected use patterns for eugenol based on the proposed conditions of use (see Table 2.1 and Table 3.1):

1. Streamside, lakeside, and shoreside (marine/estuarine) use: 5 to 100-gallon tanks containing 25 to 100 mg L^{-1} eugenol discharged instantaneously to surface waters.

2. Use on electroshocking boats: 50 to 100-gallon tanks containing 25 to 100 mg L^{-1} eugenol discharged instantaneously to ponds and lakes.

3. Use on marine boats: 25 to 250-gallon tanks containing 15 to 40 mg L^{-1} eugenol discharged instantaneously to marine waters (e.g., ocean, estuary)

For all the direct exposure scenarios, the disposal pathway for eugenol is directly to the surface water. Therefore, the PEC_{sw}[7] values were calculated. For all exposure scenarios, the initial PEC_{sw} values $\left(PEC_{sw}^{dilution}\right)$ only considered dilution or dispersion. These values were then refined for sorption to organic matter $\left(PEC_{sw}^{dilution + sorption}\right)$ if additional refinement was necessary (e.g., if the PEC exceeded relevant effect values). PEC_{sw} values were generally calculated as the daily (24-hr) average concentration because there is no limitation on the frequency of use of AQUI-S® E10% (i.e., it could be used every day). Thus, the 24-hr average PEC_{sw} represents a realistic worst-case scenario.

The assessment of the indirect exposure scenario for AQUI-S® E10% was performed by estimating the concentration of eugenol residues in the tissues of treated fish that may be consumed by scavenger or predator species after release or disposal of the fish at the site of use. If a treated fish dies following sedation and handling and its carcass is disposed of at the specific use site (e.g., streamside, lakeside), a non-target scavenger species could consume the fish carcass and be exposed to residues of eugenol in fish tissues. Although unlikely based on the labeled use directions regarding animal recovery[8], if a treated live fish remains lethargic after

[7] PEC_{sw} is a general term for all surface water PEC values. Specific PEC_{sw} values were calculated for each exposure pathway and were named according to the exposure pathway and the environmental fate processes evaluated (e.g., $PEC_{stream}^{dilution}$ is the PEC_{sw} value in the water column for streamside use and accounts for dilution only).
[8] The label states that fish should not be left unattended during sedation and recovery and further instructs users to monitor fish until completely recovered.

release into the waterbody (e.g., stream, lake), this could result in a higher likelihood of predation by non-target predator species.

Each exposure scenario and the calculation of PEC_{sw} values or tissue concentrations are discussed in the following sections.

7.1 Streamside use of eugenol

At freshwater streamside sites, 5 to 100-gallon tanks containing 25 to 100 mg L^{-1} eugenol are used to sedate fish to handleable. After use, the sedative solution is discharged to the waterbody at the site of use.

7.1.1 Conceptual Model

For this exposure scenario, two temporal scales were evaluated. In the short-term scale, it was assumed that AQUI-S® E10% is diluted by flowing water over a 1-min mixing period and a corresponding PEC_{sw} value [$PEC_{stream}^{dilution}$ (1-min)] was calculated. In the longer-term scale, the 2-hr PEC_{sw} values were initially calculated assuming only dilution occurs based on average annual streamflow [$PEC_{stream}^{dilution}$ (2-hr)] This conceptual model is shown in Figure 7.1. The initial 2-hr PEC_{sw} values were then refined by assuming instantaneous partitioning to organic matter [$PEC_{stream}^{dilution+sorption}$ (2-hr)]. It is reasonable to assume sorption occurs because disposal via this exposure scenario would likely occur in shallow water where overlying waters are in equilibrium or near-equilibrium with the sediment.

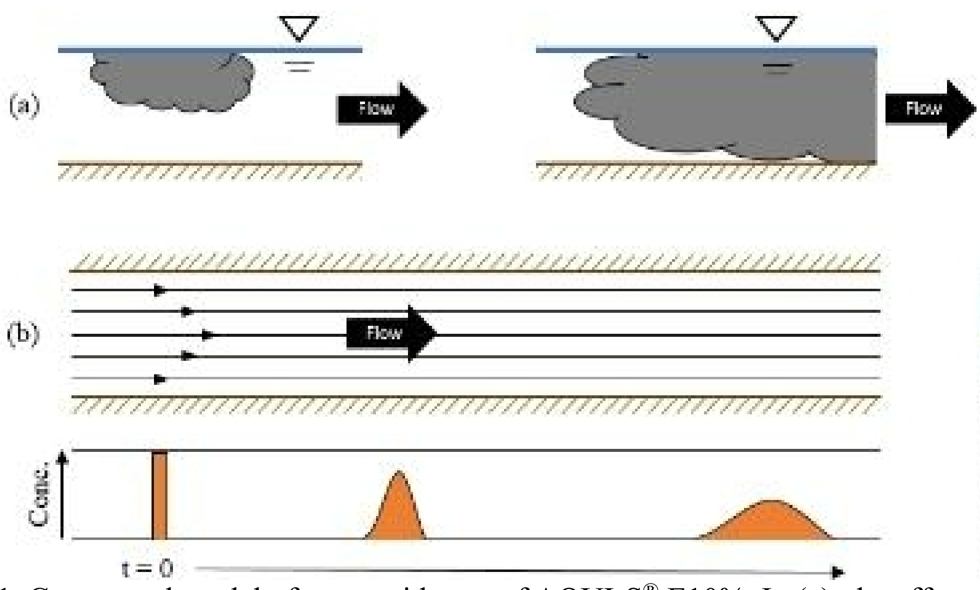

Figure 7.1. Conceptual model of streamside use of AQUI-S® E10%. In (a), the effect of advection and dispersion on vertical mixing are shown at two time points. The eugenol plume expands vertically from the surface, beginning at the site of disposal. In (b), the effect of advection and dispersion on horizontal mixing are shown. As eugenol is transported downstream, the concentration reduces while the horizontal area of the plume increases.

7.1.2 PEC_{sw} values for the streamside use of eugenol

The initial concentration of eugenol in the stream is calculated using Eq. 7.1.

$$C_1V_1 = C_2V_2 \qquad \text{Eq. 7.1}$$

where:

C_1	=	Concentration of eugenol in immersion solution	[mg L^{-1}]
V_1	=	Volume of the immersion solution tank	[L]
C_2	=	Concentration of eugenol in the waterbody	[mg L^{-1}]
V_2	=	Volume of the mixing zone	[L]

Eq. 7.1 can be rearranged and explicit variables substituted yielding Eq. 7.2

$$PEC_{stream}^{dilution} = \frac{C_{eugenol}V_{tank}}{V_{sw} + V_{tank}} \qquad \text{Eq. 7.2}$$

where:

$PEC_{stream}^{dilution}$	=	Initial predicted environmental concentration of eugenol in the stream mixing zone	[mg L^{-1}]
$C_{eugenol}$	=	Concentration of eugenol in the immersion solution tank	[mg L^{-1}]
V_{tank}	=	Volume of the immersion tank	[L]
V_{sw}	=	Volume of the mixing zone in the waterbody	[L]

The $\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution}}$ values obtained from Eq. 7.2 were refined to account for adsorption $\left(\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution+sorption}}\right)$ using equilibrium partitioning theory, which is commonly used by the US EPA in the Tier 1 Rice model.[32] The Tier 1 Rice model is a conservative screening model, and because the partitioning theory used in the Tier 1 Rice model is applicable to any natural system, it was appropriate for this use.

$$PEC_{stream}^{dilution+sorption} = PEC_{stream}^{dilution} \times \frac{1}{1 + \Gamma_{sw} f_{oc} K_{oc}} \qquad \text{Eq. 7.3}$$

where:

$PEC_{stream}^{dilution+sorption}$	=	Predicted environmental concentration of dissolved eugenol in the stream mixing zone	[mg L^{-1}]
Γ_{sw}	=	Ratio of sediment mass to water volume	[kg L^{-1}]
f_{oc}	=	Weight fraction of organic carbon in sediment	[kg kg^{-1}]
K_{oc}	=	Organic carbon normalized soil-water partition coefficient	[L kg^{-1}]

The value of Γ_{sw} was determined by Eq. 7.4:

$$\Gamma_{sw} = \frac{m_{sed}}{V_{sw}} = \frac{d_{sed}A_{sw}\rho_b}{d_{sw}A_{sw}} \qquad \text{Eq. 7.4}$$

where:

m_{sed}	=	Mass of sediment	[kg]
V_{sw}	=	Volume of water column	[m^3]
d_{sed}	=	Depth of sediment	[m]
A_{sw}	=	Area of water column	[m^2]
ρ_b	=	Bulk density of sediment	[kg m^{-3}]
d_{sw}	=	Depth of the water column	[m]

For this assessment, the values of d_{sed} and ρ_b were selected as 0.05 m and 1,300 kg_{wwt} m^{-3}, respectively. These values were obtained from the European Medicines Agency (EMA) Guidance for Veterinary Medical Products[33] and are traditionally accepted by CVM as conservative values.

Substituting Eqs. 7.2 and 7.4 into Eq. 7.3 yields the final equation:

$$PEC_{stream}^{dilution+sorption} = \frac{C_{eugenol}V_{tank}}{V_{sw}+V_{tank}} \times \frac{d_{sw}}{d_{sw}+d_{sed}\rho_b f_{oc}K_{oc}} \qquad \text{Eq. 7.5}$$

The values of $C_{eugenol}$, V_{tank}, f_{oc}, and K_{oc} are known or assumed based on reputable documentation (e.g., EMA). Values of V_{sw} and d_{sw} are not known and are expected to vary considerably across the US. Thus, to determine the degree of sorption, conservative estimates of the physical characteristics of the water column were necessary. The depth and flow rate of rivers/streams in the US were obtained from the United States Geological Survey (USGS) National Water Information System (NWIS).[34] A total of 153,925 sites were identified with gage height and/or streamflow data. From these sites, 36,533 annual mean gage height values and 565,375 annual mean streamflow data were obtained.[9] The 10th, 50th, and 90th percentile gage height and streamflow are shown in Table 7.1. For this assessment, the 10th percentile gage height and streamflow were used because these values minimize the effect of dilution and would, therefore, result in conservative estimates of the PEC_{sw} values. The 10th percentile gage height was used as the value of d_{sw}.

Table 7.1. Gage height and streamflow quantile values obtained from the USGS NWIS database.[34]

Quantile	Gage height (d_{sw}) [ft]	Streamflow (Q_{sw}) [ft^3 s^{-1}]
10th	1.687	6.62
50th	4.998	152.5
90th	28.407	2286.0

[9] Note, the number of gage height and streamflow values are not correlated 1:1 with the number of site values because each site could return one or more annual mean values. Thus, some sites may be overrepresented in the gage height and streamflow data.

To evaluate the effect of dilution and sorption on the exposure concentration, the PEC_{sw} values for a 1-min $\left[PEC_{stream}^{dilution}\ (\text{1-min})\right]$ and a 2-hr dilution period $\left[PEC_{stream}^{dilution}\ (\text{2-hr})\right]$ were calculated for each potential combination of $C_{eugenol}$ and V_{tank} proposed for streamside use. Based on the $PEC_{stream}^{dilution}$ (2-hr) value, a refined PEC_{sw} value corresponding to a 2-hr sorption period $\left[PEC_{stream}^{dilution+sorption}\ (\text{2-hr})\right]$ was also calculated (see Eqs. 7.3, 7.4, and 7.5). The PEC_{sw} values corresponding to 1-min dilution $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution}}(\text{1-min})\right]$, 2-hr dilution $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution}}(\text{2-hr})\right]$, and 2-hr dilution + sorption $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution\ +\ sorption}}(\text{2-hr})\right]$.

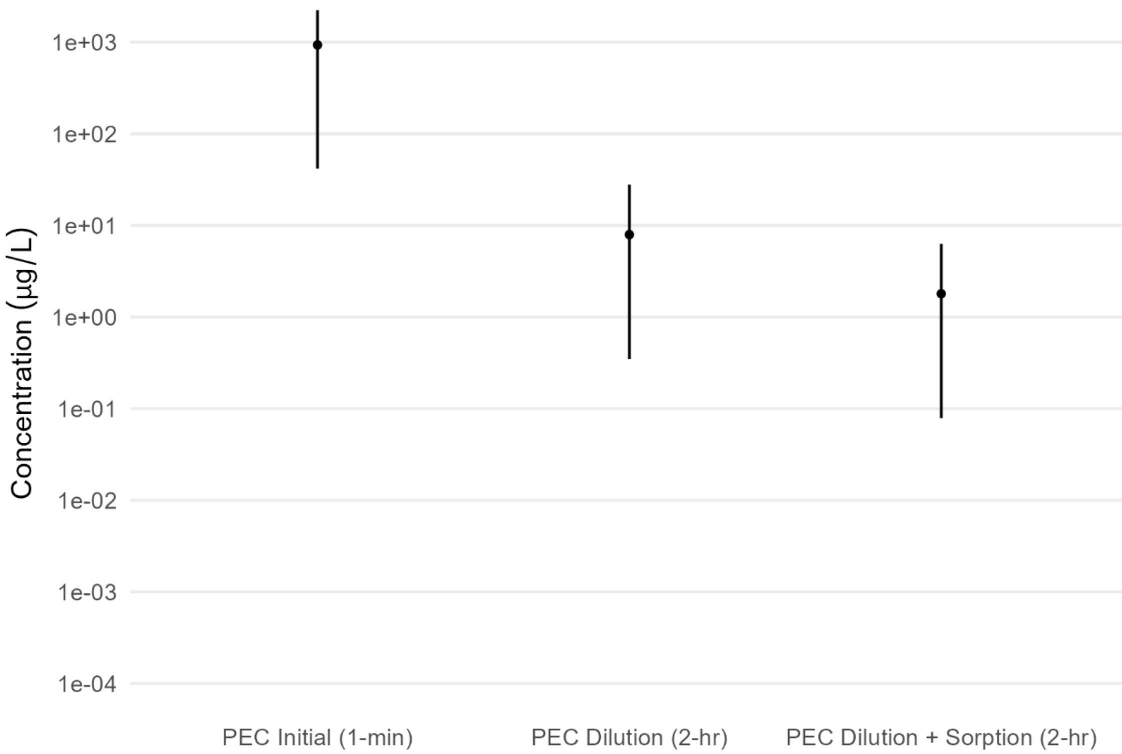

Figure 7.2. Predicted environmental concentrations (PEC_{sw}) (y-axis) in streamside surface waters following 1-min dilution $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution}}(\text{1-min})\right]$, 2-hr dilution $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution}}(\text{2-hr})\right]$, or 2-hr dilution and equilibrium sorption $\left[\mathrm{PEC}_{\mathrm{stream}}^{\mathrm{dilution\ +\ sorption}}(\text{2-hr})\right]$. Median values are shown as points, the range of values are shown as vertical lines.

7.2 Use of eugenol on boats

7.2.1 Conceptual model

In this exposure scenario, electroshocking boats on freshwater waterbodies (e.g., lakes and rivers) are assumed to discharge holding tanks (50 – 100 gal), containing residues of eugenol (25 – 100 mg L^{-1}), directly to the waterbody. Similarly, marine boats on saltwater waterbodies (e.g., oceans and estuaries) are assumed to discharge holding tanks (25 – 250 gal), containing residues of eugenol (15 – 40 mg L^{-1}), directly to the waterbody. For these evaluations, the effect of dispersion and diffusion were quantitatively calculated to determine the concentration of eugenol at 24- and 48-hr post-discharge.

In this assessment, a plume of eugenol arising from the disposal of the holding tanks was assumed to be small at the time of disposal. As time progresses, the plume is transported within the waterbody (lake, ocean, or estuary) via advection. Due to advection and dispersion, the plume grows, both vertically and horizontally. This behavior is shown in Figure 7.3. This conceptual model only considers the effects of advection and dispersion on resultant PEC_{SW} values. Other environmental processes (e.g., wave action, wind mixing) would be expected to reduce the concentration of eugenol in the plume and/or aid in greater dispersion of the plume but are not quantified herein. Therefore, this approach is expected to produce conservative estimates of aquatic exposure.

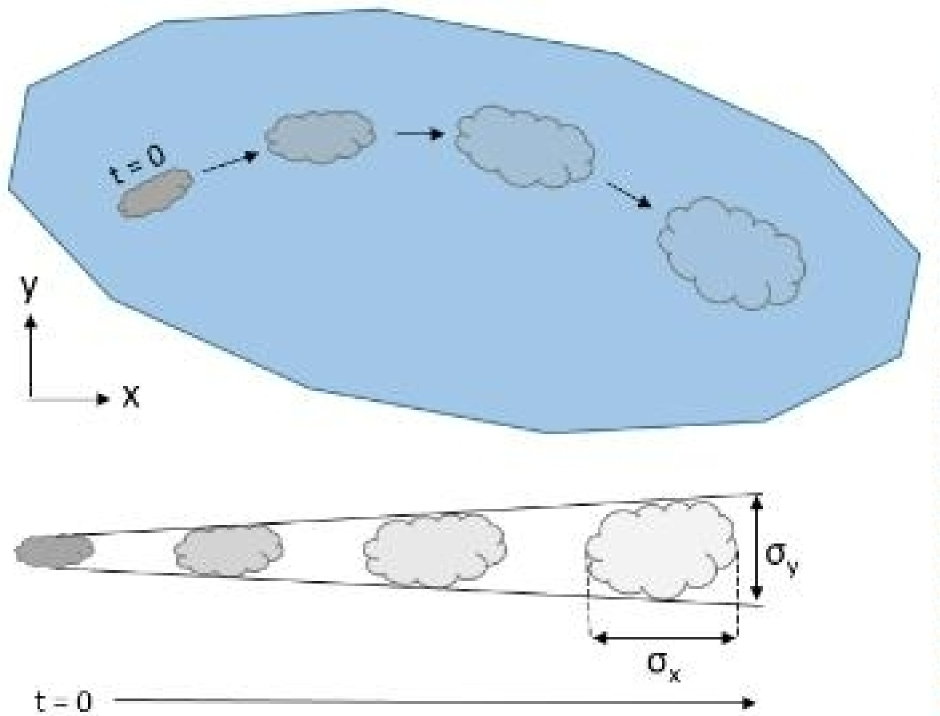

Figure 7.3. Conceptual model for use of eugenol on boats. The effect of mixing (via advection and dispersion) is shown for a plume of eugenol disposed of instantaneously at time 0 (t = 0). As time progresses, the plume is transported within the waterbody and the size of the plume increases in both the major (σ_x) and minor (σ_y) axes (vertical dispersion is minimal over short time periods (i.e., hours to days), but would also increase the size of the plume).

7.2.2 PEC_{sw} values for the use of eugenol on electroshocking boats

To determine the concentration of eugenol 24-hr post-disposal, the initial size of the plume was needed. For this purpose, it was assumed that the entire holding tank volume is disposed of into the lake and forms a 1-ft (0.305 m) deep circular plume. For a 100-gal tank, the largest volume, the initial area of the plume is given by Eq. 7.6.

$$\sigma_o^2 = \frac{V}{d} \sim \frac{100\ gal}{1\ ft} = 1.242\ m^2 \qquad \text{Eq. 7.6}$$

where:

σ_o^2	=	Initial horizontal size of the eugenol plume	[m^2]
V	=	Volume of AQUI-S® E10% solution	[gal]
d	=	Assumed initial depth of the plume	[ft]

Due to dispersion and diffusion, as time passes, the plume will spread vertically and horizontally as well as move around the lake (Figure 7.3). Therefore, to quantify the spread of the plume, the effect of dispersion and diffusion were calculated. Advection and dispersion in lakes has been studied and modeled by Carter and Okubo (1965)[35] and Peeters et al. (1996)[36] among others. Their research resulted in predictive models for vertical and horizontal turbulent dispersion.

Vertical dispersion is often minimal, especially over short (e.g., 1 day) timespans. However, an estimate can still be made using Eq. 7.7.

$$\sigma_z \sim (2E_z t)^{1/2} \qquad \text{Eq. 7.7}$$

where:

σ_z	=	Vertical size of the eugenol plume	[m]
E_z	=	Vertical diffusivity of eugenol	[m^2s^{-1}]
t	=	The time elapsed	[s]

As a conservative estimate of the vertical diffusivity, a lower limit value of 10^{-6} m^2 s^{-1} was used herein.[37] Thus, using Eq. 7.7 after a 24-hr period the expected vertical size of the cloud is 0.52 m.[10]

For the horizontal advection/dispersion, the shear-diffusion model developed by Carter and Okubo (1965)[35] is appropriate [as discussed in Peeters et al. (1996)[36]]. This model assumes the plume develops in an elliptical fashion and that the growth in both the major and minor axes are equal.

$$\sigma_{xy}^2 = 2\left[4A_1^2t^2 + \frac{1}{3}A_1A_2t^4\right]^{1/2} \qquad \text{Eq. 7.8}$$

where:

σ_{xy}	=	Horizontal size of the eugenol plume	[m^2]
A_1	=	Factor accounting for diffusive shear	[m^2s^{-1}]
A_2	=	Factor accounting for velocity shear	[m^2s^{-3}]
t	=	The time elapsed	[s]

Peeters et al. (1996)[36] and Schwarzenbach et al. (2005)[37] provide estimates of A_1 and A_2 for various waterbodies. Using these values and Eq. 7.8, the volume of the plume is calculated at t =

[10] The vertical size of the plume was calculated as follows. Initially the vertical size of the plume is 0.305 m, which is the assumed initial depth of the plume following disposal. This corresponds to when t in Eq. 7.7 is equal to 12.9 hr ($t = \sigma_z^2 \div 2E_z$). To find the size of the plume 24-hr later, the value of t was incremented by the back-calculated value (12.9-hr). Thus, Eq. 7.7 is solved for σ_z when $t = 24 + 12.9$ = 36.9 hrs. To account for this time offset (t_{offset}), the vertical size should be solved for $t + t_{offset}$.

24-hr (86,400 s) and at t = 48-hr (172,800 s) for all combinations of treatment concentration (ranging from 25 to 100 mg L^{-1}) and holding tank volume (50 to 100 gal). The associated PEC_{SW} was calculated using the equation $C_1V_1 = C_2V_2$, where C_1 is the treatment concentration, V_1 is the volume of the holding tank, V_2 is the size of the plume after 24- or 48-hr, and C_2 is the concentration of eugenol in that plume (i.e., PEC_{SW}). Results for the highest treatment concentration and largest holding tank volume at t = 24-hr are shown in Table 7.2, below.

Table 7.2. Predicted environmental concentrations (PEC_{sw}, which is equivalent to C_2) in waterbodies for the use of eugenol on electroshocking boats at the maximum proposed treatment concentration (C_1 = 100 mg L^{-1}) and maximum tank volume (V_1 = 100 gal) after accounting for advection and dispersion over a 24-hr period.[36, 37]

Waterbody	A_1 [m^2s^{-1}]	A_2 [10^{-10} m^2s^{-3}]	σ_z (t) [m]	$\sigma_{xy}(t)^2$ [10^4 m^2]	V_2 [10^4 m^3]	C_2 [µg L^{-1}]
Alpnachersee	0.03	0.12	0.52	1.16	0.60	6.34
Vitznauersee						
1	0.03	1.3		1.99	1.03	3.68
2	0.027	1		1.70	0.87	4.33
3	0.016	3		1.97	1.01	3.73
Lac de Neuchâtel	0.18	1.8		7.92	4.08	0.93
Urnersee						
1	0.09	0.9		3.96	2.04	1.85
2	0.05	40		12.31	6.35	0.60
Lake Ontario						
ED1	0.05	0.4		2.11	1.09	3.47
ED5	0.16	1		6.52	3.36	1.13
ED3	0.02	0.006		0.70	0.36	10.52
Mikawa Bay	0.066	290		37.78	19.48	0.19
Banana River	0.09	0.2		3.32	1.71	2.21
Off Southern California	0.11	700		75.73	39.05	0.10
Pacific (1000 m depth)	0.04	-0.001		1.38	0.71	5.32
Lake						
Calm	0.04	1		2.21	1.14	3.32
Windy	0.2	100		39.16	20.19	0.19
Ocean	0.1	500		61.05	31.48	0.12

Alpnachersee, Vitznauersee, and Urnersee are part of Lake Lucerne, Switzerland.
A1 = Factor accounting for diffusive shear in the waterbody; A2 = Factor accounting for velocity shear in the waterbody; σ_z = Vertizal size of the eugenol plume; σ_{xy} = Horizontal size of the eugenol plume; V_2 = Volume of the plume after 24 hours; C_2 = Concentration of eugenol in the plume after 24 hours

During and beyond the mixing period evaluated, concentrations of eugenol would be expected to decline further due to degradation, sorption to dissolved and particulate organic matter, and other environmental processes such as wave action. As noted in both the mineralization (Appendix B.2) and adsorption (Appendix B.3) studies conducted to support the approval of AQUI-S® E10%, eugenol rapidly dissipates in surface water.[25, 29] Therefore, the PEC_{sw} values in Table 7.2

above are likely overestimates of the concentrations that would be expected to occur in the environment. However, to remain conservative and evaluate the reasonable worst-case effects of the use of AQUI-S® E10% on boats, the PEC_{sw} values reported in Table 7.2 are used in the risk characterization contained herein (see Section 9).

7.2.3 PEC_{sw} values for the use of eugenol on marine boats

The estimation of PEC_{sw} values for marine[11] boats follows the same method used for electroshocking boats in freshwater (Section 7.2.2, above). For the marine boat scenario, it was assumed that the entire holding tank volume is disposed of into the lake and forms a 1-ft (0.305 m) deep circular plume. The initial area of the plume (σ_0^2) is given by Eq. 7.6 and, for the maximum tank volume (250-gal), is equal to 3.10 m^2.

To estimate the vertical dispersion of the plume, Eq. 7.7 is used again. Estimates at both 24-hr and 48-hr after disposal were calculated, however only the 24-hr results are shown below as they are more conservative (higher PEC_{sw}). After a 24-hr period the expected vertical size of the cloud is 0.52 m. Similarly, the shear-diffusion model developed by Carter and Okubo (1965)[35] (Eq. 7.8) and the values of A_1 and A_2 are again used to estimate the area of the plume. Results at t = 24-hr are shown in Table 7.3. See Section 7.2.2 for additional details regarding the shear-diffusion model.

Table 7.3. Predicted environmental concentrations (PEC_{sw}, which is equivalent to C_2) in waterbodies for the use of eugenol on marine boats at the maximum proposed treatment concentration (C_1 = 40 mg L^{-1}) and maximum tank volume (V_1 = 250 gal) after accounting for advection and dispersion over a 24-hr period.[36, 37]

Waterbody	A_1 [m^2s^{-1}]	A_2 [10^{-10} m^2s^{-3}]	σ_z (t) [m]	$\sigma_{xy}(t)^2$ [10^4 m^2]	V_2 [10^4 m^3]	C_2 [µg L^{-1}]
Alpnachersee	0.03	0.12		1.16	0.60	6.34
Vitznauersee						
1	0.03	1.3		1.99	3.68	3.68
2	0.027	1		1.70	4.33	4.33
3	0.016	3		1.97	3.73	3.73
Lac de Neuchâtel	0.18	1.8		7.92	4.08	0.93
Urnersee						
1	0.09	0.9		3.96	1.85	1.85
2	0.05	40	0.52	12.31	0.60	0.60
Lake Ontario						
ED1	0.05	0.4		2.11	3.47	3.47
ED5	0.16	1		6.52	1.13	1.13
ED3	0.02	0.006		0.70	10.53	10.52
Mikawa Bay	0.066	290		37.78	19.47	0.19
Banana River	0.09	0.2		3.32	1.71	2.21
Off Southern California	0.11	700		75.73	39.04	0.10

[11] The marine boat use scenario is also applicable to use in estuarine waters such as bays and large tidal rivers.

Pacific (1000 m depth)	0.04	-0.001		1.38	0.71	5.32
Lake						
Calm	0.04	1		2.21	3.32	3.32
Windy	0.2	100		39.16	0.19	0.19
Ocean	0.1	500		61.05	31.47	0.12

Alpnachersee, Vitznauersee, and Urnersee are part of Lake Lucerne, Switzerland.
A1 = Factor accounting for diffusive shear in the waterbody; A2 = Factor accounting for velocity shear in the waterbody; σ_z = Vertizal size of the eugenol plume; σ_{xy} = Horizontal size of the eugenol plume; V_2 = Volume of the plume after 24 hours; C_2 = Concentration of eugenol in the plume after 24 hours

As in the freshwater electroshocking boat analysis, concentrations of eugenol would be expected to decline further due to degradation, sorption to dissolved and particulate organic matter, and other environmental processes, such as wave action. However, to remain conservative and evaluate the reasonable worst-case effects of the use of AQUI-S® E10% on boats, the PEC_{sw} values reported in Table 7.3 are used in the risk assessment contained herein (see Section 9).

7.3 Lakeside and Shoreside use of eugenol

7.3.1 Conceptual Model

At freshwater lakeside sites, it is assumed that 5 to 100-gallon tanks containing 25 to 100 mg L^{-1} eugenol are used to sedate fish to handleable. In marine shoreside sites (e.g., oceanside, estuarine rivers, bays, and harbors), it is assumed that 5 to 100-gallon tanks containing 25 to 40 mg L^{-1} eugenol will be used to sedate fish to handleable. After use, the sedative solution is discharged to the waterbody at the site of use. As the lakeside use pattern encompasses the range of volumes and concentrations expected for the shoreside use pattern, only a single conservative analysis was conducted to cover both scenarios using the maximum tank volume (i.e., 100 gallons) and maximum eugenol concentration (i.e., 100 mg L^{-1}).

7.3.2 PEC_{sw} values for the lakeside and shoreside use of eugenol

An estimate of the concentration of eugenol after disposal at lakeside and shoreside sites is possible via application of a slightly modified version of approach used in Section 7.2. As in the boat scenarios, the shear-diffusion model developed by Carter and Okubo (1965)[35] (Eq. 7.8) and the values of A_1 and A_2 were used to estimate the area of the plume. Unlike the boat scenarios (Section 7.2), however, which allowed the eugenol plume to develop in an elliptical fashion, the lakeside and shoreside scenarios only allowed the eugenol plume to develop in a half-ellipse because the lake and shore are bounded by land. Thus, to conservatively estimate the amount of dilution that would occur in the lakeside and shoreside scenarios, the plume areas determined from Eq. 7.8 were divided by 2. In addition, based on proximity to lakeside and shoreside sediment at the site of introduction, the effect of sorption was considered using the same equilibrium partitioning theory employed for the streamside calculations presented in Section 6.1.2.

For the lakeside and shoreside scenarios, it was assumed that the entire tank volume is disposed of into the lake or ocean immediately at the shoreline and forms a 0.25-ft (0.076 m) deep circular plume. The initial area of the plume (σ_0^2) is given by Eq. 7.6 and, for the maximum tank volume (100-gal) is equal to 4.97 m^2. To estimate the vertical dispersion of the plume, Eq. 7.7 was used again. After a 24-hr period, the expected vertical size of the plume is 0.42 m. The results at t = 24-hr without sorption considered (C_2) and with sorption considered ($C_{2\text{-sorption}}$) are shown in Table 7.4, below.

Table 7.4. Predicted environmental concentrations without and with sorption (PEC_{sw}, which is equivalent to C_2 and $C_{2\text{-sorption}}$) in waterbodies for the use of eugenol lake- and shore-side at the maximum proposed treatment concentration (C_1 = 100 mg L^{-1}) and maximum tank volume (V_1 = 100 gal) after accounting for advection and dispersion over a 24-hr period.[36, 37]

Waterbody	A_1 [m^2s^{-1}]	A_2 [10^{-10} m^2s^{-3}]	σ_z (t) [m]	$\sigma_{xy}(t)^2$ [10^4 m^2]	V_2 [10^4 m^3]	C_2 [µg L^{-1}]	$C_{2\text{-sorption}}$ [µg L^{-1}]
Alpnachersee	0.03	0.12		1.16	0.24	15.46	3.07
Vitznauersee							
1	0.03	1.3		1.99	0.42	8.99	1.79
2	0.027	1		1.70	0.36	10.56	2.10
3	0.016	3		1.97	0.42	9.10	1.81
Lac de Neuchâtel	0.18	1.8		7.92	1.67	2.26	0.45
Urnersee							
1	0.09	0.9		3.96	0.84	4.52	0.90
2	0.05	40		12.31	2.60	1.45	0.29
Lake Ontario							
ED1	0.05	0.4		2.11	0.45	8.47	1.68
ED5	0.16	1	0.42	6.52	1.38	2.75	0.55
ED3	0.02	0.006		0.70	0.15	25.68	5.11
Mikawa Bay	0.066	290		37.78	7.98	0.47	0.09
Banana River	0.09	0.2		3.32	0.70	5.40	1.07
Off Southern California	0.11	700		75.73	16.00	0.24	0.05
Pacific (1000 m depth)	0.04	-0.001		1.38	0.29	12.97	2.58
Lake							
Calm	0.04	1		2.21	0.47	8.11	1.61
Windy	0.2	100		39.16	8.28	0.46	0.03
Ocean	0.1	500		61.05	12.90	0.29	0.06

Alpnachersee, Vitznauersee, and Urnersee are part of Lake Lucerne, Switzerland.
A1 = Factor accounting for diffusive shear in the waterbody; A2 = Factor accounting for velocity shear in the waterbody; σ_z = Vertizal size of the eugenol plume; σ_{xy} = Horizontal size of the eugenol plume; V_2 = Volume of the plume after 24 hours; C_2 = Concentration of eugenol in the plume after 24 hours; $C_{2\text{-sorption}}$ = Concentration of eugenol in the plume after 24 hours, accounting for losses via sorption

As noted above, environmental processes such as wave action in shoreside or lakeside settings could serve to rapidly mix eugenol with clean water, further diluting the plume over short time periods. PEC_{sw} values for the lakeside and shoreside scenarios range from 0.24 to 25.68 µg L^{-1}.

7.4 Summary of PEC_{sw} values

Final PEC_{sw} values were calculated for streamside, lakeside and shoreside, electroshocking boat, and marine boat uses of AQUI-S® E10%. The range of PEC_{sw} values for each use pattern and exposure period (i.e., 2, 24, or 48 hours) are shown in Table 7.5 and reflect a distribution of results based on varying combinations of treatment tank volume and concentration ranges presented in Table 3.1.

Table 7.5. Summary of acute predicted environmental concentrations in surface water (PEC_{sw}) for all evaluated exposure scenarios

Scenario	PEC_{sw} [µg L^{-1}]		Section
	Median	Range	
Streamside[a] (Dilution; 2-hr)	7.93	(0.35 – 27.84)	7.1.2
Streamside[a] (Dilution + Sorption; 2-hr)	1.80	(0.082 – 6.30)	
Electroshocking Boats (Dispersion; 24-hr)	0.86	(0.012 – 10.5)	7.2.2
Electroshocking Boats (Dispersion; 48-hr)	0.22	(0.002 – 3.99)	
Marine Boats (Dispersion; 24-hr)	0.70	(0.007 – 10.5)	7.2.3
Marine Boats (Dispersion; 48-hr)	0.18	(0.001 – 3.99)	
Lakeside and Shoreside (Dispersion; 24-hr)	1.10	(0.003 – 25.68)	7.3.2
Lakeside and Shoreside (Dispersion + Sorption; 24-hr)	0.22	(0.00060 – 5.11)	
Lakeside and Shoreside (Dispersion; 48-hr)	0.26	(0.0005 – 8.91)	
Lakeside and Shoreside (Dispersion + Sorption; 48-hr)	0.07	(0.00014 – 2.30)	

[a] PEC_{SW} values for streamside scenarios were calculated based on the assumption of a 2-hr sorption and dilution period. PEC_{sw} values at 24- and 48-hr would be substantially lower than the value shown here.

7.5 Exposure to Non-target Scavenger or Predator Species

An additional exposure pathway was evaluated to address the potential for treated fish or treated fish carcasses to be released or disposed of at the site of treatment (e.g., streamside, lakeside), thus serving as a route of dietary exposure to non-target scavenger or predator species (i.e., carnivorous birds, fish, and mammals). This pathway is considered incidental and is expected to be infrequent and sporadic.

To determine the potential exposure concentrations in the tissues of treated fish, three residue chemistry studies were consulted. Two residue depletion studies and a marker residue study in rainbow trout (*Oncorhynchus mykiss*) provide the maximum tissue concentrations of eugenol, as well as the time course for eugenol residue depletion following exposure. These data are summarized below.

7.5.1 Maximum Tissue Residue

Under Study No. AEH-11-EUGNL-01, a US Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) study, the maximum eugenol concentration in fillet tissue of treated rainbow trout (weighing 167 – 404 g) was determined. The fish were exposed to several different concentrations of AQUI-S® E10% (10% eugenol w/w) over a range of exposure durations to determine which treatment condition resulted in the highest tissue residue levels. Rainbow trout were exposed to 50 – 1,000 mg L^{-1} AQUI-S® E10% (equivalent to 5 – 100 mg L^{-1} eugenol) for 5 – 240 minutes (mins) in 17°C water. At the termination of each exposure period, five replicate fish were sampled to determine fillet tissue concentrations, and eugenol concentrations in the tissues were measured via high performance liquid chromatography (HPLC). The test conditions that resulted in the maximum eugenol concentration in fillet tissue were then used in a residue depletion study (described in Section 7.5.2).

The eugenol concentrations in the fillet tissues for all exposure conditions tested ranged from 6.87 – 77.3 mg kg^{-1} with mean concentrations ranging from 8.05 ± 1.3 to 62.0 ± 10 mg kg^{-1}. The maximum tissue eugenol concentration of 77.3 mg kg^{-1} (mean 62.0 ± 10 mg kg^{-1}) resulted from exposures to 100 mg L^{-1} AQUI-S® E10% (~ 10 mg kg^{-1} eugenol) for 240 mins. While this study also characterized residue levels in fish exposed to the proposed dosage level (i.e., 1,000 mg kg^{-1} AQUI-S® E10%, or ~ 100 mg kg^{-1} eugenol) for durations ranging from 15 to 90 mins, the highest residue concentration of 77.3 mg kg^{-1}, which resulted from exposure to 100 mg L^{-1} AQUI-S® E10% (~ 10 mg kg^{-1} eugenol) for 240 mins was selected for use in this risk analysis as a measure of conservatism (see Section 9.1.2). Therefore, eugenol concentrations in fillet tissues at termination of exposure to 100 mg L^{-1} AQUI-S® E10% (~ 10 mg kg^{-1} eugenol) for 240 mins are presented in Table 7.6.

Table 7.6. Eugenol concentration in fillets of rainbow trout exposed to 100 mg L^{-1} AQUI-S® E10% (~ 10 mg L^{-1} eugenol) for various durations

Exposure Duration (mins)	Mean Eugenol Tissue Concentration (mg kg^{-1}) ± SD	Minimum – Maximum Eugenol Concentration (mg kg^{-1})
5	15.4^{a} ± 3.4	11.5-20.5
10	23.8^{b} ± 5.0	16.6-28.6
15	28.6^{b} ± 4.3	21.7-32.2
30	50.1^{c} ± 4.0	45.9-55.4
60	58.2^{c} ± 10.0	49.8-74.8
120	53.6^{c} ± 9.2	41.3-62.8
240	62.0^{c} ± 10.0	50.4-77.3

Means with the same superscript letter are not significantly different ($p>0.05$)

It is important to note that, in this treatment group, there was no significant difference in mean eugenol concentrations in the fillet tissues when exposed for between 30 to 240 mins[12] (see Table 7.6). However, the mean and maximum eugenol concentrations were significantly lower when exposed for 5, 10, or 15 mins.

[12] These data indicate that the tissue concentration reaches equilibrium in approximately 30 mins.

7.5.2 Tissue Residue Depletion

Three tissue residue depletion studies were conducted by exposing rainbow trout to 100 mg L^{-1} AQUI-S® E10% (~ 10 mg L^{-1} eugenol) for 60 mins. The three studies are summarized below support a residue depletion rate in freshwater finfish.

1) Study No. AEH-11-EUGNL-01 (USGS, UMESC)
   Rainbow trout were exposed to 100 mg L^{-1} AQUI-S E10% (~ 10 mg L^{-1} eugenol) for 60 mins in 17°C water and then moved to clean water and fillet tissues were sampled from 5 replicate fish for eugenol residues at 30, 60, 120, 240, 360, 720, and 1,440 mins. Eugenol concentrations were measured via HPLC.

2) Study No. AEH-11-EUGNL-02 (USGS, UMESC)
   A total residue depletion study was conducted where rainbow trout were exposed to 10 mg L^{-1} ^{14}C-eugenol for 60 mins in 17 °C water and skin-on fillet tissue was sampled from 6 replicate fish at 0, 30, 60, and 120 mins after exposure. ^{14}C-eugenol was measured via liquid scintillation counting (LSC) and eugenol concentrations were also measured via HPLC.

3) Study No. AEH-13-EUGNL-05 (USGS, UMESC)
   A marker residue study was conducted where rainbow trout were exposed to 100 mg L^{-1} AQUI-S E10% (~ 10 mg L^{-1} eugenol) for 60 mins in 9°C water and then placed in clean water and skin-on fillet tissues were sampled from 16 replicate fish at 0, 15, 30, 90, and 150 mins after exposure. Eugenol concentrations were measured via HPLC-ultraviolet spectroscopy.

Eugenol concentrations in fillet tissue at various post-exposure sampling times (0 – 1,440 mins) are reported for all three studies in Table 7.7 below. In all studies, the eugenol concentrations in fillet tissue declined rapidly within 30 to 60 mins after treatment. Under Study No. AEH-11-EUGNL-02, depletion half-lives of 26.25 mins for ^{14}C-eugenol and 19.97 mins for eugenol were estimated.

Table 7.7. Eugenol concentrations (mg kg-1) in fillet or skin-on fillet tissue of rainbow trout after exposure to 100 mg L^{-1} AQUI-S E10% or 10 mg L^{-1} ^{14}C-eugenol[a] for 60 mins

Time after exposure (mins)	Mean Eugenol Tissue Concentration (mg kg^{-1}) ± SD		
	10 mg L^{-1} ^{14}C-eugenol for 60 mins (Study No. AEH-11-EUGNL-02)	**100 mg L^{-1} AQUI-S E10% for 60 mins (Study No. AEH-11-EUGNL-01)**	**100 mg L^{-1} AQUI-S E10% for 60 mins (Study No. AEH-13-EUGNL-05)**
0	38.77 ± 7.9		48 ± 6.5
15			29 ± 3.8
30	12.42 ± 1.7	19.7 ± 5	19 ± 3.4
60	7.41 ± 2.0	10.5 ± 4	
90			4.5 ± 1.1
120	3.95 ± 0.81	4.09 ± 2.3	
150			1.9 ± 0.65
240	1.77 ± 0.47	0.51 ± 0.55	

360		0.44 ± 0.29	
720		0.06 ± 0.032	
1440		0.01 ± 0.015	

[a] 100 mg L^{-1} AQUI-S® E10% is a 10% w/w solution, equivalent to ~10 mg L^{-1} eugenol

Based on the residue chemistry data, the highest residues of eugenol in the tissues of fish (i.e., 77.3 mg kg^{-1}) resulted from fish treated with 100 mg L^{-1} AQUI-S® E10% (~10 mg L^{-1} eugenol) for a duration of 240 mins (see Table 7.6). Eugenol tissue residues for fish treated in accordance with the current proposed conditions of use (i.e., 100 mg L^{-1} eugenol for a duration leading to sedation to handleable condition (typically achieved within 1 – 3 mins)) are expected to be far lower. This maximum residue concentration reflects a worst-case scenario for tissue residues and tissue residues resulting from the proposed conditions of use indicated to achieve sedation to a handleable condition are expected to be far less. Based on Study AEH-11-EUGNL-01, the most closely aligned test conditions (i.e., 1,000 mg L^{-1} AQUI-S® E10% (~100 mg L^{-1} eugenol) for 15 mins) results in fish tissue residue concentrations of 23.3 – 35.2 mg kg^{-1} (mean 29.1 mg kg^{-1} ± 5.7). Furthermore, based on residue depletion data, eugenol residues in fish tissues are expected to rapidly decline to less than half the initial concentration within 30 mins post exposure and decline further over the following hours (see Table D.2), resulting in minimal exposure to non-target scavenger or predator species unless consumed shortly (1-2 hours) after treatment.

8 Effects assessment

This section summarizes all available data on the acute and chronic effects of eugenol on aquatic organisms from publicly funded studies, studies owned by AQUI-S New Zealand, and the published literature. Effects data for algae, invertebrates, and fish were reported in order to represent three trophic levels of the aquatic ecosystem. Executive summaries for publicly funded studies are provided in Appendix A, while summaries of studies owned by AQUI-S New Zealand are provided in Appendix B.

8.1 Acute effects for freshwater organisms

Acute effects are usually reported based on short-term exposures (e.g., ≤ 96-hr) to high concentrations of xenobiotics, with exposed organisms typically monitored for mortality. Several studies report the acute effects of eugenol on various algae and fish species, as well as *Daphnia magna*, an aquatic invertebrate. The results of these studies are summarized below.

One publicly-funded study, conducted following GLP standards and in accordance with OECD Guideline 201,[38] reported a 72-hr median effects concentration (EC_{50}) for cell density in a freshwater green algae (*Pseudokirchneriella subcapitata*) as 14 mg L^{-1}.[39] Additional effects data reported for freshwater algae species is provided in Table 8.1, below.

Table 8.1. Summary of the median effects concentrations (EC_{50}) for freshwater algae species exposed for 72-hr

Algae Species	Effect	72-hr EC_{50} [mg L^{-1}]
Pseudokirchneriella subcapitata	Cell density	14.0[39]
Desmodesmus subspicatus	Cell density	40.8[40]
Scenedesmus subspicatus[13]	Biomass	22.0[41]

Following OECD Guideline 202,[42] Brougher et al. (2013)[43] reported 24- and 48-hr EC_{50} values for immobilization in *D. magna*[14] as >1.7 mg L^{-1} and 0.74 mg L^{-1}, respectively. At 24 hours, no inhibitory or other effects were observed on *D. magna* at or below the 0.86 mg L^{-1} test concentration; however, immobilization was observed in 5 of the 20 *D. magna* exposed at the highest test concentration (i.e., 1.7 mg L^{-1}). Several additional 48-hr EC_{50} values for immobilization in *D. magna* have been reported in the published literature. These 48-hour EC_{50} values, ranging between 1.11 and 1.9 mg L^{-1}, support those reported by Brougher et al. (2013).[43] See Table 8.2 for all available data regarding effects of eugenol in *D. magna*. All of the effects values reported for *D. magna* are consistent, increasing the reliability of the data obtained from Brougher et al. (2013).[43] In addition, the data obtained from Brougher et al. (2013)[43] is also the lowest effects value reported (i.e., 0.74 mg L^{-1}). Therefore, this value is also conservative.

Table 8.2. Summary of the median effects concentrations (EC_{50}) for *Daphnia magna* exposed to eugenol for 24- or 48-hr

Effect	24-hr EC_{50} [mg L^{-1}]	48-hr EC_{50} [mg L^{-1}]
Immobilization	>1.7[43]	0.74[43]
		1.11[44]
		1.13[16]
Mortality	NR	1.9[41]

NR = not reported

Several studies report acute effects in various freshwater fish species. One study owned by AQUI-S New Zealand [Brougher et al. (2015)],[45] was conducted following GLP standards and in accordance with OECD Guideline 203,[46] reported a 96-hr median lethal concentration (LC_{50}) in *Pimephales promelas* (fathead minnows) as 19 mg L^{-1}. Additional 96-hr LC_{50} values for *Oncorhynchus mykiss* (rainbow trout) and *Danio rerio* (zebrafish) are also reported in Table 8.3, below.

Table 8.3. Summary of the median lethal effects concentrations (LC_{50}) for freshwater fish exposed to eugenol for 96-hr

Fish Species	96-hr LC_{50} [mg L^{-1}]
***Pimephales promelas* (fathead minnow)**	19[45]
	24[47]
***Oncorhynchus mykiss* (rainbow trout)**	5.6[41]
	>10[44]
***Danio rerio* (zebrafish)**	13[41]
	13[16]

[13] The specific algae species identified in EFSA (2012), *Scenedesmus subcapicatus*, is now often classified as *Desmodesmus subspicatus*.
[14] It is important to note that immobilization in *Daphnia magna* is equivalent to a mortality endpoint.

8.2 Acute effects for saltwater organisms

Limited data are available in the published literature, regarding acute effects of eugenol to saltwater organisms. All of the available data are reported in Table 8.4, below.

Table 8.4. Summary of median lethal concentrations (LC_{50}) for saltwater organisms exposed to eugenol for 24- or 48-hr

Species	Duration	LC_{50} [mg L^{-1}]
Fenneropenaeus indicus **(Indian prawn)**	24-hr	5.0[48]
Lateolabrax maculatus **(spotted sea bass)**	24-hr	19.7[49]
Barbus sharpeiy **(binni)**	48-hr	22.6[50]
Ochlerotatus caspius **(seaside mosquito)**	48-hr	5.6[51]

8.3 Acute effects for mammals

To determine the potential effects in non-target scavenger or predator species, acute oral toxicity studies were consulted. Acute toxicity data in mammals obtained from public literature are summarized in Table 8.5 below. These data are typically used to evaluate potential effects to humans that consume fish with residues of eugenol but can also be used for the purpose of the current evaluation. It is important to note that subchronic and chronic data in mammals was not used herein because the exposure to non-target scavenger or predator species is expected to be a rare, sporadic event resulting in an acute exposure. To be conservative, the lowest no observed effect level (NOEL) of 200 mg kg^{-1} for dogs was selected for use in the risk analysis in Section 9.1.2 below.

Table 8.5. Acute oral toxicity of eugenol to mammals

Species	LD_{50} (mg kg bw^{-1})	NOEL (mg kg bw^{-1})	Findings
Rat	1930	N/A	Mortality, decrease righting reflex, dyspnea, visceral organ congestion and inflammation[52]
Rat	2680	N/A	Mortality, liver discoloration and mottling[53]
Rat	>2000	N/A	Mortality, dose levels at 150, 250, 500, 1000, and 2000 mg kg bw^{-1} [54]
Guinea pig	2130	N/A	Mortality, decreased activity[55]
Mouse	3000	N/A	Mortality, decreased activity[55]
Mouse	>3000	N/A	Mortality, dose levels at 180, 375, 750, 1500, and 3000 mg kg bw^{-1} [54]
Dog	500	200	Ataxia, vomiting, nausea, liver congestion, hemorrhage in the stomach and duodenum[56]

LD_{50} = Median lethal dose
NOEL = No observed effect level

8.4 Chronic effects

Chronic effects generally result from long-term exposures (e.g., 21-d) and may be reported as effects on reproduction, growth, or survival. For eugenol, chronic toxicity data are limited. Nevertheless, based on the conditions of use for AQUI-S® E10%, the exposure patterns for both freshwater and saltwater are expected to result in short duration events due to rapid dissipation in the water column (e.g., mixing, dilution, dispersion, sorption, and transport of the drug in surface waters). As a result, it is expected that any exposure in a particular area will be short-term (e.g., ≤ 48-hr). See Section 7 for additional information regarding the expected exposure of AQUI-S® E10%. Therefore, chronic exposures and any associated chronic effects, are not expected and are not discussed further in this EA.

8.5 Derivation of aquatic predicted no effects concentration (PNEC)

Acute predicted no effect concentrations (PNECs) are determined by dividing experimentally derived acute effect endpoints by an appropriate assessment factor (AF), which is intended to cover uncertainties within the data such as intra- and inter-laboratory and species variation, extrapolation from results obtained from laboratory studies to the field, and from acute to chronic toxicity. As described in CVM GFI 166,[2] the AF will vary depending on the species tested and specific study conducted.

Based on all available data, as described in Section 8 above, the species resulting in the most sensitive acute effects endpoint for both freshwater and saltwater organisms was *D. magna*, an aquatic invertebrate (i.e., 24- and 48-hr EC_{50} for immobilization as >1.7 mg L^{-1} and 0.74 mg L^{-1}, respectively). The appropriate AF for an acute *D. magna* study is 1,000, as specified in CVM GFI 166.[2] This AF may be reduced if additional information permits. For example, the AF may be reduced if information suggests that the chemical is rapidly degraded and not repeatedly administered, which could result in a chronic exposure. Due to the conditions of use of AQUI-S® E10% and expected exposure patterns resulting from these conditions of use (i.e., direct release to surface waters wherein mixing, dilution, dispersion, sorption, and transport occur), it is expected that any exposure in a particular area will be short-term (e.g., ≤ 48-hr). As a result, chronic exposures, and any associated chronic effects, are not expected. Therefore, the AF used to estimate 24-hr and 48-hr acute PNEC values for *D. magna* were reduced by a factor of 10 (i.e., AF = 1,000 ÷ 10 = 100) because it was not necessary to account for extrapolation from acute to chronic toxicity in the AF. Furthermore, three additional data sources reported EC_{50} values for *D. magna* in the same range as those used to derive the PNEC (see Table 8.2), supporting the reliability of the endpoints used derive the acute PNECs. The PNEC was determined using Eq. 8.1:

$$PNEC = \frac{Effect\ Endpoint}{AF} \qquad \text{Eq. 8.1}$$

Using Eq. 8.1, an AF = 100, and acute effect endpoints corresponding to the 24-hr and 48-hr EC_{50} for immobilization in *D. magna*, the acute PNECs were calculated as 0.017 mg L^{-1} (17 µg L^{-1}) and 0.0074 mg L^{-1} (7.4 µg L^{-1}). Although additional acute PNECs could be derived for other freshwater and saltwater species (i.e., algae, fish, shrimp, mosquitoes), the effect concentrations for these species are substantially greater than that for *D. magna*. As a result, the acute PNECs for these species would be higher and would not be as conservative as that derived for *D. magna*.

In addition, the applied AF used to derive the acute PNEC for *D. magna* accounts for organisms that may be up to 100 times more sensitive. Therefore, the acute PNEC for *D. magna* is considered conservative and should be protective of other freshwater and saltwater organisms.

9 Analysis and risk characterization

The median and range of PEC_{sw} values derived for each exposure scenario and the 24- and 48-hr acute PNEC values are shown in below in Figure 9.1. The horizontal dashed and solid lines correspond to the 24- and 48-hr acute PNECs derived in Section 8.5, above. Only three scenarios (i.e., the unrefined streamside and lakeside and shoreside scenarios incorporating only dilution or dispersion) show maximum PEC_{SW} values above the corresponding PNECs, indicating that predicted exposure levels exceed concern levels for aquatic organisms. However, the maximum PEC_{SW} value for all three of those scenarios falls below corresponding PNECs once sorption is considered.

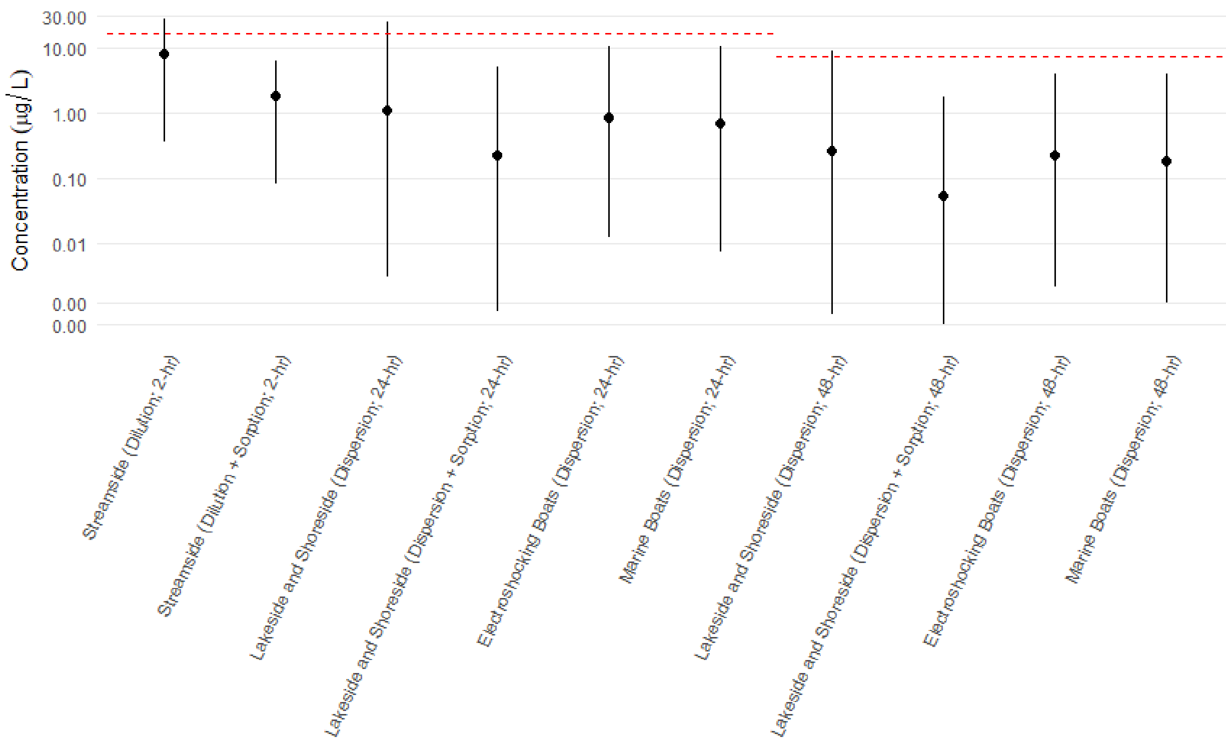

Figure 9.1. Comparison of predicted environmental concentrations in surface water (PEC_{SW}; y-axis) for all exposure scenarios (x-axis) to the lowest acute predicted no effects concentrations (PNEC) determined for 24- and 48-hr exposure durations for *Daphnia magna*. Median values are shown as points, the range of values are shown as vertical lines.

9.1 Characterization of Risk

The risk characterization was conducted based on the risk quotient (RQ) approach. In the RQ approach, the most refined PEC_{sw} value in Section 7 are compared to PNECs derived in Section 8. An RQ less than 1.0 indicates that there is no expectation of significant effects to the environment because the concentration needed to elicit an effect is greater than the concentration expected in the environment from the use of AQUI-S® E10%. In contrast, an RQ equal to or greater than 1.0 indicates that effects to the environment could occur from the use. RQs were calculated for each exposure pathway using equation .

$$RQ = \frac{PEC}{PNEC} \quad \text{Eq. 9.1}$$

9.1.1 Risks to Aquatic Organisms from Direct Exposure Scenarios

The median and maximum PEC_{sw} values for each direct exposure scenario evaluated, and the corresponding RQs based on the 24-hr and 48-hr acute PNEC values for *D. magna* (i.e., 17 and 7.4 µg L^{-1}, respectively), are shown in Table 9.1. To derive the RQs for each scenario, the 24-hr PNEC was compared to 24-hr PEC_{sw} values and the 48-hr PNEC was compared to the 48-hr PEC_{sw} values. In the absence of a 2-hr PNEC, RQs for streamside use were derived by comparing the 24-hr PNEC to the 2-hr PEC_{sw} values. This is considered conservative because the 24-hour PNEC is expected to be protective of effects occurring before and up to 24 hours (i.e., a 2-hr PNEC would be expected to be higher than a 24-hr PNEC).

Table 9.1. Summary of predicted environmental concentrations (PEC_{SW}), predicted no effect concentrations (PNEC), and risk quotients (RQs) for all evaluated exposure scenarios

Scenario	**PEC_{sw} [µg L^{-1}]**		**PNEC [µg L^{-1}]**	**RQ**	
	Median	Range		Median	Maximum
Streamside[a] (Dilution; 2-hr)	7.93	0.35 – 27.84	17	0.47	1.64
Streamside[a] (Dilution + Sorption; 2-hr)	1.80	0.082 – 6.30	17	0.11	0.37
Electroshocking Boats (Dispersion; 24-hr)	0.86	(0.012 – 10.5)	17	0.051	0.62
Electroshocking Boats (Dispersion; 48-hr)	0.22	(0.002 – 3.99)	7.4	0.030	0.54
Marine Boats (Dispersion; 24-hr)	0.70	(0.007 – 10.5)	17	0.041	0.62
Marine Boats (Dispersion; 48-hr)	0.18	(0.001 – 3.99)	7.4	0.024	0.54
Lakeside and Shoreside (Dispersion; 24-hr)	1.10	(0.003 – 25.68)	17	0.06	1.51
Lakeside and Shoreside (Dispersion + Sorption; 24-hr)	0.22	(0.00060 – 5.11)	17	0.01	0.30
Lakeside and Shoreside (Dispersion; 48-hr)	0.26	(0.0005 – 8.91)	7.4	0.04	1.20
Lakeside and Shoreside (Dispersion + Sorption; 48-hr)	0.052	(9.94E-05 – 1.77)	7.4	0.0070	0.24

RQs are above 1.0 for three scenarios (i.e., unrefined streamside, lakeside, and shoreside uses). However, once sorption is considered for these scenarios (dilution + sorption), RQs are all below 1.0, and therefore, significant environmental impacts are not expected from the proposed use of eugenol (i.e., streamside, lakeside, shoreside, in electroshocking boats on freshwater lakes, and in boats on estuarine/marine waters).

9.1.2 Risks to Scavenger or Predator Species from Indirect Exposure Scenario

The risk analysis for secondary poisoning of scavenger or predator species was conducted by comparing the overall maximum eugenol concentrations in fillet tissue with the lowest NOEL for mammals. The maximum eugenol concentration of 77.3 mg kg^{-1} found in tissues of fish treated with 100 mg L^{-1} eugenol for 240 mins (see Section 7.5.1 above) was 2.5-times below the lowest NOEL of 200 mg kg^{-1} for dogs (see Section 8.3 above), meaning that expected exposures to scavenger or predator species are conservatively 2.5 times less than the experimentally derived no effect level in mammals. Thus, effects in non-target scavenger or predator species from consumption of fish containing residues of eugenol are not expected. Eugenol concentrations in tissues of treated fish are also expected to rapidly decline within 30 – 60 mins and continue to decline over time, further reducing the potential exposure. In addition, based on labeled use instructions for animal recovery, treated fish are expected to be placed in a container with clean water immediately after handling and prior to release back into the waterbody. This recovery step is also expected to further reduce eugenol residues in treated fish tissues that scavenger or predator species could be exposed to.

In consideration of the information reviewed and analyses performed, significant environmental impacts to non-target scavenger or predator species are not expected from the proposed action.

10 Cumulative impacts

There are no other products containing eugenol approved in the US for use in terrestrial or aquatic animals. Therefore, there is no expectation of cumulative impacts from the approval and use of AQUI-S® E10%.

11 Alternatives to the proposed action

The only alternative to the proposed action is the “no action” alternative, which would be the failure to approve the new animal drug application (NADA) for AQUI-S® E10% (eugenol immersion solution). However, based on our analysis in this EA, we do not believe that significant environmental impacts will occur from this action; therefore, the “no action” alternative was eliminated from consideration.

12 Conclusions

This EA evaluated the proposed field-use of AQUI-S® E10% for sedation to handleable condition in all freshwater and saltwater finfish through an exposure assessment and risk characterization of three direct pathways of introduction into the environment: (1) disposal to streamside, lakeside, and shoreside use sites; (2) disposal to lakes or ponds following use on electroshocking boats; and (3) disposal to marine/estuarine water bodies following use on marine boats. Once appropriate refinements were incorporated (e.g., sorption to sediment), RQ values for all exposure scenarios were less than 1.0, indicating that there is no expectation of adverse effects to water-dwelling organisms from these use patterns. It should be noted that the RQ values derived herein are considered to be conservative because the PEC_{SW} values were calculated using only dilution or dispersion, and in some cases sorption. It is known that eugenol readily dissipates in surface water due to environmental fate processes such as degradation, which would substantially reduce environmental concentrations over short (i.e., < 48-hr) time periods. Further, mixing processes, such as wave action in large waterbodies like a lake, bay tidal river or ocean, were not accounted for in this analysis. These processes would quickly reduce concentrations of eugenol due to dispersion, dilution, and sorption to organic matter. In addition, the potential for secondary poisoning of non-target scavenger or predator species via consumption of treated fish tissues was evaluated, and no significant environmental effects were identified. Thus, it can be concluded that significant environmental effects are not expected from the proposed use of AQUI-S® E10% (eugenol) immersion solution.

13 List of agencies and persons consulted

This EA was prepared with input and assistance from members of the Environmental Branch in the Office of New Animal Product Evaluation (ONAPE) in FDA's Center for Veterinary Medicine (CVM).

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Appendix A Executive Summaries of Publicly Funded Studies

A.1 Brougher DS, Martin KH, Gallagher SP, and Krueger HO. 2013. Eugenol: A 48-hour static acute toxicity test with the cladoceran (Daphnia magna). Project no. 738A-101A.[43]

The objective of this study was to determine the acute effects of eugenol on the cladoceran, *Daphnia magna*, during a 48-hr exposure period under static test conditions. The study was conducted according to procedures outlined in the OECD Test Guideline 202.[42] Daphnids were exposed to a geometric series of five test concentrations and a negative control (dilution water) for 48 hours under static conditions. Four replicate test chambers were maintained in each treatment and control group, with five daphnids in each test chamber, for a total of 20 daphnids per concentration. Nominal test concentrations, based on the results of exploratory range finding toxicity data, were selected as 0.13, 0.25, 0.50, 1.0 and 2.0 mg L^{-1}. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning and end of the test. Mean measured concentrations of eugenol ranged from 0.11 to 1.7 mg L^{-1}. The 48-hr median effective concentration (EC_{50}) value was 0.74 mg L^{-1}, with a 95% confidence interval of 0.43 to 1.7 mg L^{-1}. The no-immobility concentration and the no observed effect concentration (NOEC) were both 0.43 mg L^{-1}.

A.2 Jacobs AM, Martin KH, and Porch JR. 2013. Eugenol: A 72-Hour Toxicity Test with the Freshwater Alga (Pseudokirchneriella subcapitata). Wildlife International Project No.: 738P-101.[39]

The objective of this study was to determine the toxicity of eugenol to the freshwater alga, *Pseudokirchneriella subcapitata*, over a 72-hr exposure period under static test conditions. The study was conducted according to procedures outlined in the OECD Test Guideline 201.[38] *P. subcapitata* was exposed to a geometric series of five test concentrations and a negative (culture medium) control under static conditions for 72 hours. Three replicate test chambers were maintained in each treatment, and six replicate test chambers were maintained for the control group. Nominal test concentrations, based on an exploratory range-finding test, were selected as 1.0, 2.6, 6.4, 16 and 40 mg L^{-1}. Measured test concentrations were reported as 0.98, 2.6, 6.3, 16, and 39 mg L^{-1}, respectively. Effects data was reported based on the measured test concentrations. The 72-hr 10^{th}, 20^{th}, and median effect concentration values (EC_{10}, EC_{20}, and EC_{50}, respectively) for cell density were 6.9, 8.7 and 14 mg L^{-1}, respectively. The 72-hr no observed effect concentration (NOEC) value for cell density was 0.98 mg L^{-1}. The 72-hr EC_{10}, EC_{20}, and EC_{50} values for growth rate were 14, 18 and 27 mg L^{-1}, respectively. The 72-hr NOEC value for growth rate was 2.6 mg L^{-1}.

Appendix B Executive Summaries of Studies Owned by AQUI-S New Zealand

B.1 Brougher DS, Keller K, Gallagher SP, and Martin KH. 2015. Eugenol: A 96-hour static-renewal acute toxicity test with the fathead minnow (Pimephales promelas). Project no. 739A-101A.[45]

The objective of this study was to determine the acute effects of eugenol on the fathead minnow (*Pimephales promelas*) during a 96-hr exposure period under static-renewal test conditions. The study was conducted according to procedures outlined in the OECD Test Guideline 203.[46] Fathead minnows were exposed to a geometric series of six test concentrations and a negative control (dilution water) for 96 hours under static-renewal conditions. The fish were transferred to newly-prepared test solutions at approximately 48 hours. One replicate test chamber was maintained in each treatment and control group, with 10 fish in each test chamber, for a total of 10 fish per concentration. Nominal test concentrations, based on exploratory range-finding toxicity data, were selected as 4.0, 6.0, 9.0, 13, 20 and 30 mg L^{-1}. Test concentrations were measured in samples of test water collected from each treatment and control group at the beginning of the test, before and after renewal at the approximate mid-point of the test (approximately 48 hours), and at the end of the test. Results of the analyses were used to calculate mean measured test concentrations. Mean measured concentrations ranged from 3.2 to 35 mg L^{-1}. The 96-hr median lethal concentration (LC_{50}) value was 19 mg L^{-1}, with a 95% confidence interval of 13 to 35 mg L^{-1}. The 96-hr no-mortality concentration was 13 mg L^{-1} and the 96-hr no observed effect concentration (NOEC) was 7.3 mg L^{-1}. Apparent loss of test substance was observed in the old test solutions at 96 hours. Therefore, the LC_{50}, the no mortality concentration, and NOEC were also calculated for the 48-hour time interval. The 48-hr LC_{50} value was 20 mg L^{-1}, with a 95% confidence interval of 13 to 35 mg L^{-1}. The 48 hour no-mortality concentration and NOEC were unchanged and were 13 mg L^{-1} and 7.3 mg L^{-1}, respectively.

B.2 Robinson E and Schaefer EC. 2015. [^{14}C] Eugenol: Aerobic mineralization in surface water. Wildlife International Project No. 739E-102.[29]

The degradation of [^{14}C] eugenol was studied in biotic and abiotic surface water systems at two concentrations differing by a factor of 5. Known quantities of radiolabeled [^{14}C] eugenol were applied to test chambers at nominal concentrations of ~6 µg L^{-1} for low concentration and ~30 µg L^{-1} for high concentration treatments, respectively, and incubated at approximately 20 °C for 60 days. At appropriate sampling intervals, biotic and abiotic treatment test chambers as well as reference chambers (positive controls used to verify the viability of biotic test systems) were acidified and transferred to a flow-through system designed to capture ^{14}C volatile organics (ethylene glycol traps) and evolved $^{14}CO_2$ (1.5 N KOH traps). After at least 12 hours, the test chambers and traps were removed from the flow-through system. The surface water was analyzed for total radioactivity, and traps were analyzed for captured ^{14}C volatile organics and $^{14}CO_2$ by liquid scintillation counting (LSC). Aliquots of select samples of surface water were prepared using solid phase extraction (SPE). The quantity of radioactivity in SPE extracts were determined by LSC. The radioactive distribution of parent test substance and transformation products in select SPE extracts were analyzed by high performance liquid chromatography with a radio flow detector (HPLC-beta ram). Disappearance of the [^{14}C] eugenol from the biotic test systems was attributed to mineralization (evolved $^{14}CO_2$).

For the biotic low treatment (~ 6 µg L^{-1}), an increase in % applied radioactivity (AR) in the traps was observed over the 60-d study. At the ~5-min interval, <1% of the AR was identified in the traps. By day 14, there was an increase to 46.4% of AR in the traps; 46.3% AR was due to mineralization. By day 60, 69.6% of AR was captured in the traps; 46.4% was due to mineralization. The abiotic low treatment had an overall increase in mineralization ranging from 0.4% AR at the ~5-min interval to 13.0% AR at day 60.

For the biotic high treatment (~ 30 µg L^{-1}), an increase in total traps was also observed. At the ~5-min interval, <1% of the AR was identified in the traps. At day 7 an increase to 39.9% AR was identified in the traps; 31.3% AR was due to mineralization. By day 60, 52.2% AR was measured in the traps and was entirely due to mineralization. The abiotic high treatment also had an overall increase in mineralization ranging from 0.2% at ~5 min interval to 14.7% at day 60.

Mean mass balance recoveries (sum of total traps + total radioactivity of dosed surface water) were calculated for ~5 minute, and 2-, 7-, 14-, 30-, 40-, 51-, and 60-d intervals. Mass balance recoveries were classified as sampling intervals following: 1) the acidification technique (~5 minute through day 30 intervals; test chambers were opened and acidified resulting in low mass balance), and 2) modified procedure (day 40 through 60 intervals; test chambers were unopened during acidification).

Reference test chambers were analyzed as viability controls to ensure the microbial activity of the biotic inoculum. The average $^{14}CO_2$ produced for the biotic replicates of ^{14}C benzoic acid dosed at 25 µg L^{-1} was >50% (67.6% AR) by day 7 and remained >50% throughout the end of the study.

Kinetic analysis of the observations was not conducted. Therefore, rate constants and half-lives were not determined.

B.3 Schaefer E and Ponizovsky A. 2015. Eugenol: Adsorption/Desorption Characteristics in Representative Soils. Wildlife International Project No.: 739E-101.[25]

Isotherms of eugenol adsorption and desorption were obtained for five representative soils. The soils (silt loam, sandy loam, loamy sand, clay loam, and clay) contained 0.8 % to 4.3% organic carbon, 10.5% to 43.9% clay, and 9360 to 12090 mg kg^{-1} free iron oxides-hydroxides. The pH of the soils (measured in 0.01 M $CaCl_2$) ranged from 5.3 to 7.7.

Testing was performed using radiolabeled [^{14}C] eugenol. Eugenol was stable in aqueous 0.01 M $CaCl_2$ solution with pH adjusted to 5.0 and 6.9 after 24-hr equilibration. Transformation of eugenol was observed in aqueous 0.01 M $CaCl_2$ suspensions of non-sterilized soils. Usage of γ-sterilized soils with filter-sterilized 0.01 M $CaCl_2$ solution and test vessels sterilized by autoclaving decreased but did not eliminate transformation of eugenol.

Isotherms of eugenol adsorption were obtained within initial eugenol concentration ranging from 1.1-1.2 µg L^{-1} to approximately 55.4-57.7 µg L^{-1} resulting in adsorption equilibrium concentrations from 0.2-0.4 µg L^{-1} to 10-24 µg L^{-1}. The adsorption and desorption isotherms were close to linear. Average values of adsorption coefficients (K_d^{ads}) in the test systems were calculated by fit of the adsorption isotherms and ranged from 8.51 ± 0.30 L kg^{-1} for the clay soil (0.8% OC) to 49.6 ± 0.71 L kg^{-1} for the clay loam soil (4.3% OC). The values of organic carbon normalized adsorption coefficient (K_{oc}^{ads}) ranged from 626 L kg^{-1} to 1441 L kg^{-1}.

Average values of eugenol desorption coefficients (K_d^{des}) ranged from 76.5 ± 3.13 L kg^{-1} for the loamy sand soil to 167 ± 1.64 L kg^{-1} for the sandy loam soil. The values of organic carbon normalized desorption coefficient (K_{oc}^{des}) ranged from 2479 L kg^{-1} to 8359 L kg^{-1}.