APPENDIX B – CHAPTER 3 AQUATIC RISK ASSESSMENT

1.0 Introduction

This chapter considers the hazards, exposure, and relative risks for each herbicide used by WSDOT to fish and aquatic macroinvertebrates. Direct application of the herbicides to the aquatic environment does not occur within the scope of WSDOT roadside management; however, exposure is possible through drift during application, runoff during rainfall events, and leaching through the soils into ground water sources. The goal of this chapter, as established in the original EIS, is to determine the potential for WSDOT's herbicide use to adversely affect the aquatic biota (WSDOT 1993).

1.1 Objectives

The objectives of this Aquatic Risk Assessment are as follows:

- To describe the toxicity of the selected herbicides currently used by WSDOT to freshwater and marine aquatic organisms, where data are available.
- To identify sensitive species that may be impacted in different regions.
- To characterize exposure by identifying actual and potential exposure pathways, taking into account environmental fate and transport through both physical and biological means.
- To characterize the risk or threat to environmental receptors potentially exposed to the selected herbicides.

1.2 Overview

This aquatic ecological risk assessment uses the methods applied in the original WSDOT EIS (WSDOT 1993), incorporating current literature since 1993, and present application rates used by WSDOT. The waters involved in the fate and transport of chemicals for this assessment are considered those immediately adjacent to or near roadsides. Herbicides, which can reach receiving waters, would most likely dissipate through dilution, degradation, and sequestering by a variety of processes. Roadside management activities include the use of herbicides where appropriate to control vegetation at the pavement edge, where noxious or nuisance weeds occur, and/or for unobstructed views of road signage and other safety issues. Impact to the aquatic environment could occur from drift during application, runoff during precipitation events, and/or leaching from herbicide-contaminated groundwater into surface waters.

For this assessment, the potential for each herbicide to accumulate in and cause toxicity to freshwater and estuarine/marine fish and aquatic invertebrates are discussed where data are available. Although Washington State supports several ESA-listed aquatic species, these species are not often those that are used for toxicity testing. Thus, the assessment of hazards to aquatic species did not focus on a single receptor but instead on summarized data for several species, as available from the literature. In revising this EIS, we examined literature compiled on the herbicides for their aquatic toxicity and fate, focusing particularly on literature developed since 1993. Often, we found no new data on the chemicals despite using the most sophisticated literature search engines, including Aquatic Sciences and Fisheries Abstracts, Biosis, Agricola, Extoxnet, and U.S. EPA product registration documents.

Much of the toxicity information cited remains from the older literature as the revised search did not, in several cases, identify new information. In the final assessment of relative risk, the most sensitive species tested was used to estimate relative risk from WSDOT's herbicide use.

In considering the aquatic toxicity data, it is important to recognize some standard toxicological terminology. Results of studies are typically expressed in terms of a no observable effect level (NOEL); the lowest observable effects level (LOEL), EC_{50}, LC_{50} and the LD_{50}. The NOEL is that concentration at which no effect is observed and is generally determined from a statistical evaluation of an empirical dose-response curve for a given chemical, and a given endpoint being measured. The LC_{50} and LD_{50} values are those concentrations and doses, respectively, which would be projected to cause 50% mortality in the exposed population(s). The "EC_{50}" value is the projected concentration that would be expected to elicit an effect in 50% of an exposed population. The LC_{50} and EC_{50} values are used for qualitative predictions of toxicity and for the establishment of requirements for product use limitation or further testing. These values are important to use in comparison to herbicide accumulation within the aquatic environment.

In the assessment of toxicity of herbicides, studies were identified that performed both "acute" (short term) and "chronic" (long term) tests. Our assessment focused primarily on acute studies due to the nature of herbicide use within the scope of WSDOT's roadside management; however, if chronic studies were identified, results from those studies are also reported. Acute values parallel the "once or twice a season per area" use pattern typical of WSDOT applications.

Chronic toxicity effects in aquatic organisms from pollutants may include adverse reproductive or growth effects, decreased hatching success, immunosuppression, or endocrine-mediated effects such as feminization—amongst others. None of the herbicides used by WSDOT are persistent to the extent that chronic exposures would be expected in aquatic systems. However, more research is needed to ascertain the probability of chronic herbicide exposure from WSDOT's vegetation management program. In addition, for the majority of the compounds under consideration in this assessment, little or no chronic toxicity data were available because earlier tier acute testing (for product registration) did not indicate the need for further data development. In those instances where chronic studies were available, the species or the endpoints used were not consistent among studies. Over the past ten years a significant effort has been undertaken to evaluate tissue residues in fish to determine whether chronic exposure is occurring, and/or whether some chemicals bioaccumulate. The database for residue-based effects with herbicides, however, is extremely limited. Where we found data, however, it is reported.

Acute data (short term studies) are generated by the conduct of 2-4 day long studies under carefully controlled conditions. Table 3-1 provides general risk assessment standards for fish and aquatic invertebrates based on acute toxicity LC_{50} concentrations. This guide was used as a preliminary template to gauge risks associated with project actions that are broad in scope, such as the use of herbicides by WSDOT for roadside management.

In each section, the 17 herbicide formulations in 2003 are presented first, followed by the 12 herbicide formulations examined in 2005. The herbicide formulations examined in 2003 include:

1. 2,4-D
2. Ammonium salt of fosamine
3. Bromacil/Diuron
4. Chlorsulfuron

5. Clopyralid
6. Clopyralid/2,4-D
7. Dicamba
8. Dicamba/2,4-D
9. Dicamba/MCPA
10. Dichlobenil
11. Diuron
12. Glyphosate
13. Metsulfuron Methyl
14. Picloram
15. Oryzalin
16. Sulfometuron Methyl
17. Triclopyr

The 12 herbicide formulations examined in 2005 include:

1. Bromoxynil
2. Diflufenzopyr
3. Flumioxazin
4. Fluroxypyr
5. Imazapyr
6. Isoxaben
7. Norflurazon
8. Oxadiazon
9. Pendimethalin
10. Pyraflufen
11. Sulfentrazone
12. Tebuthiuron

Table 3-1. Toxicity Classifications to Address Risk to Aquatic Organisms from Herbicide Use.

Risk Category	Fish or Aquatic Invertebrates
	Acute Concentration LC_{50} (mg/L)
Very highly toxic	<0.1
Highly toxic	0.1-1
Moderately toxic	>1-10
Slightly toxic	>10-100
Practically non-toxic	>100

2.0 Herbicide Toxicity to Aquatic Receptors

In this section we discuss the acute and chronic toxicity concentrations reported from various studies in relation to aquatic biota and the aquatic environment. Additional toxicological details are summarized in Section 3, where the exposure assessments and risk characterizations are considered. If information on adjuvants and inert ingredients was available for the specific herbicides, it was summarized; otherwise, general information on a range of adjuvants used with herbicide applications is provided at the end of the section.

2.1 2,4-D

2.1.1 Fish

Reported levels of acute toxicity LC_{50} concentrations for 2,4-D esters range from 1.0 to 100 mg/L in cutthroat trout (EXTOXNET 1996a). Ester formulations may be up to 100 times more toxic than acid forms, as described in greater detail in the exposure assessment section.

2.1.2 Aquatic Invertebrates

Concentrations of 10 mg/L of 2,4-D, administered to adult Dungeness crab for 85 days, resulted in no impact on survival; however, the same concentration administered for 96 hours at the early immature stage resulted in the death of 50 percent of the test subjects (EXTOXNET 1996a). Smaller aquatic organisms exhibited less resistance to concentrations of 2,4-D. For example, brown shrimp had a small increase in mortality at a concentration of 2 mg/L over a 48-hour exposure period (EXTOXNET 1996a).

2.2 Ammonium Salt of Fosamine

2.2.1 Fish

The 96-hour acute $LC_{50}$for Fosamine was 1,000 mg/L for rainbow trout and fathead minnow, 670 mg/L for bluegill sunfish, and 8,290 mg/L for coho salmon (Tu et al. 2001a, Swift 2002, ACOE 2003b). Acute toxicity LC_{50} after 96-hour exposure listed by BPA (2000a) were much lower, but still resulted in an overall toxicity of practically non-toxic (377 mg/L for rainbow trout, 590 mg/L for bluegill sunfish, and >200 mg/L for coho salmon).

2.2.2 Aquatic Invertebrates

Acute dermal LC_{50}s for a Fosamine Ammonium Salt formulation (42%) after a 48-hour exposure period was reported as 1,524 mg/L for *Daphnia magna*.

2.3 Bromacil/Diuron

2.3.1 Fish

Bromacil toxicity to fish and aquatic invertebrates varies from practically non-toxic to slightly toxic. It is metabolized in aquatic organisms to its debrominated analog. It does not bioaccumulate in aquatic organisms. A study looking at the embryo to juvenile life stage of fathead minnow (*Pimephales promelas*) in the lab reported a NOEL for survival and reproduction from an exposure of 29 mg/L after a 64-day period (Call *et al.* 1987 *as cited in* Jarvinen and Ankley 1999). A concentration of 93 μg/g chemical residue was reported in the body of the fathead minnow. However, the same study also reported reduced growth after an exposure of only 1.0 mg/L for the same duration (64 days). A concentration of 3 μg/g chemical residue was reported in the body of the fathead minnow.

2.3.2 Aquatic Invertebrates

No data were identified that specifically recorded the toxicity of Krovar to aquatic invertebrates.

2.4 Chlorsulfuron

The 1993 WSDOT EIS reported that, "Chlorsulfuron is practically non-toxic to the fish and aquatic invertebrates tested and does not tend to bioaccumulate," (WSDOT 1993). Review of recent literature revealed no further studies examining the aquatic toxicity of Chlorsulfuron in either fish or invertebrates.

2.5 Clopyralid

2.5.1 Fish

A study looking at the response of fish to Clopyralid reported LC_{50} concentrations of 125 mg/L for bluegill sunfish and 104 mg/L for rainbow trout (Tu et al. 2001b). In a separate report, the LC_{50} concentrations after 96-hour exposures were reported as >100 mg/L for both bluegill sunfish and rainbow trout (BPA 2000b).

2.5.2 Aquatic Invertebrates

The reported LC_{50} for *Daphnia magna* exposed to Clopyralid after a 48 hour exposure period is >100 mg/L (BPA 2000b).

2.5.3 Adjuvant and Inert Ingredient Hazards

A group of inert ingredients, polyethoxylated tallow amines, used in some commercial formulations of Clopyralid are acutely toxic to fish (Cox 1998). Adjuvants in some formulations, such as cyclohexanone, triisopropanolamine, triethylamine, may cause toxicity to terrestrial animals but their toxicity to aquatic animals has not been fully explored. The relative risks from these ingredients cannot be fully ascertained, as the specific ratio of these adjuvants within the formulations has not been reported.

2.6 Clopyralid/2,4-D

2.6.1 Fish and Aquatic Invertebrates

No specific information was identified in the literature that specifically examined the aquatic toxicity to fish or invertebrates on Curtail.

2.6.2 Adjuvant and Inert Ingredient Hazards

Information regarding aquatic environment toxicity to adjuvant and inert ingredients of Clopyralid/2,4-D commercial formulations was not reported in the compendium of literature we examined. Toxic hazards associated with the inert and adjuvant ingredients of Clopyralid could also be considered associated with the Clopryalid/2,4-D mixture.

2.7 Dicamba

2.7.1 Fish

Dicamba is considered slightly to practically non-toxic to fish. In a study looking at the response of bluegill sunfish (*Lepomis macrochirus*) to Dicamba, an LC_{50} concentration of 20,000 μg/L was reported after an exposure period of 48 hours (Hughes and Davis 1962 *as cited in* Verschueren 1983). In a study reported within Syngenta (2000), the LC_{50} concentrations for both rainbow trout and bluegill sunfish, after a 96-hour exposure period, is 135 mg/L. The USDA (1995) conferred a LC_{50} value greater than 100 mg/L for bluegill sunfish, as well as a general LC_{50} for fish that is greater than 10 mg/L.

EXTOXNET (1996) reported LC_{50} concentrations of 135 mg/L for rainbow trout and bluegill sunfish at an exposure period of 96 hours, and 35 mg/L for rainbow trout, 40 mg/L for bluegill sunfish, and 465 mg/L for carp at an exposure period of 48 hours.

2.7.2 Aquatic Invertebrates

Dicamba is considered moderately toxic to aquatic invertebrates (USDA 1989). The reported LC_{50} for *Daphnia magna* and small freshwater crustaceans, after a 48-hour exposure period, is 110 mg/L (Syngenta 2000, EXTOXNET 1996b). EXTOXNET (1996b) reported LC_{50} concentrations of >100 mg/L for grass shrimp, >180 mg/L in fiddler crab and sheepshead minnow at an exposure period of 96 hours.

A series of chronic toxicity studies involving Dicamba were performed on a variety of crustacean species (*Gammarus fasciatus, Daphnia magna, Cypridopsis vidua, Asellus brevicaudus, Palaemonetes kadiakensis, Orconectes mais*). The studies resulted in a NOEL of 100,000 μg/L for all species exposed to Dicamba for 48 hours (Sanders 1970 *as cited in* Verschueren 1983). After an exposure period of 96 hours, the crustacean species *Gammarus lacustris* had an LC_{50} concentration of 3,900 μg/L (Sanders 1969 *as cited in* Verschueren 1983).

2.8 Dicamba/2,4-D

No specific information was identified in the literature that specifically examined the aquatic toxicity to fish or aquatic invertebrates of the Veteran or Weedmaster formulations of Dicamba and 2,4-D. Toxicity can be considered similar to the Vanquish formula.

2.9 Dicamba/MCPA

No specific information was identified in the literature that specifically examined the aquatic toxicity to fish or aquatic invertebrates from Vengeance. Toxicity can be considered similar to the Vanquish formula.

2.10 Dichlobenil

2.10.1 Fish

Dichlobenil is considered slightly to moderately toxic to fish, but it does not appear to bioaccumulate. In a study looking at the response of fish (*Lepomis macrochirus*) to Dichlobenil, an LC_{50} concentration of 20,000 μg/L was reported after an exposure period of 48 hours (Wilson and Bond 1969 *as cited in* Verschueren 1983). Acute LD_{50} concentrations reported for bluegill and rainbow trout, using the same laboratory parameters, were 20.0 mg/L and 22.0 mg/L, respectively (Edwards 1977 *as cited in* Verschueren 1983). The same experiment with guppies resulted in an LC_{50} concentration of 18 mg/L (Hubert 1968 *as cited in* Verschueren 1983).

Harlequin fish (*Rasbora heteromorpha*) exhibited a range of LC10 and LC_{50} responses for a variety of exposure periods (Tooby and Hursey 1975 *as cited in* Verschueren 1983). The reported LC10 ranged from 4.3 mg/L after 24 hours to 3.3 mg/L after 96 hours, and the LC_{50} ranged from 6.2 mg/L after 24 hours and 4.2 mg/L after 96 hours.

Exposure of aquatic organisms through direct application of Dichlobenil into a nonflowing water source (i.e. pond, lake, reservoir) has the potential to create conditions yielding acute toxicity if drift or run-off are excessive (Table 3-2).

Table 3-2. Acute Toxicity Levels of Dichlobenil for Aquatic Organisms (ACOE 2003).

Species	Condition	Exposure Period (hr)	Acute Toxicity LC_{50} (mg/L)
Rainbow trout	Static, 13C (55F)	96	6.3
Bluegill sunfish	Static, 18C (64F)	96	8.3
Crustacea (Daphnia pulex)	Static, 1st instar, 15C (59F)	96	3.7
Crustacea (Grammarus lucustris)	Static, mature, 21C (70F)	96	11.0

U.S. EPA (1998) reported that levels of 0.33 mg/L (mg/L) and 0.75 mg/L may have chronic effects to fish and aquatic invertebrates, respectively.

2.10.2 Aquatic Invertebrates

A series of toxicity studies involving Dichlobenil were performed on a variety of crustacean and algae populations (Table 3-3). The reported LC_{50} concentrations for crustacean populations ranged from 8,500 to 11,000 μg/L after 96 hours of exposure and from 3,700 to 34,000 μg/L after 48 hours of exposure (Sanders 1969, Sanders 1970, Wilson and Bond 1969, Sanders and Cope 1968 *as cited in* Verschueren 1983). The reported 50 percent decrease in O_2 evolution for algae populations ranged from $9x10^4$ to $1.5x10^5$ ppb and the reported 50 percent decrease in growth ranged from $2.5x10^4$ to $6x10^4$ ppb (Walsh 1972 *as cited in* Verschueren 1983).

Table 3-3. Toxicological Response to Dichlobenil of Various Crustacean and Algae Populations.

Species	Exposure	Response	Toxicity Concentration
Chlorococcum spp.	N/A	50% decrease in O_2 evolution	9x104 ppb
	N/A	50% decrease in growth	6x104 ppb
Dunaliella tertiolecta	N/A	50% decrease in O_2 evolution	1.25x105 ppb
	N/A	50% decrease in growth	6x104 ppb
Isochrysis galbana	N/A	50% decrease in O_2 evolution	1x105 ppb
	N/A	50% decrease in growth	6x104 ppb
Phaeodactylum tricornutum	N/A	50% decrease in O_2 evolution	1.5x105 ppb
	N/A	50% decrease in growth	2.5x104 ppb
Gammarus lacustris	96 hours	LC_{50}	11,000 μg/L
Gammarus fasciatus	96 hours	LC_{50}	10,000 μg/L
Hyallella azteca	96 hours	LC_{50}	8,500 μg/L
Simocephalus serrulatus	48 hours	LC_{50}	5,800 μg/L
Daphnia pulex	48 hours	LC_{50}	3,700 μg/L
Daphnia magna	48 hours	LC_{50}	10,000 μg/L
	N/A	IC_{50}	9.8 mg/L
Cypridopsis vidua	48 hours	LC_{50}	7,800 μg/L
Asellus brevicaudus	48 hours	LC_{50}	34,000 μg/L
Palaemonetes kadiakensis	48 hours	LC_{50}	9,000 μg/L
Orconectes nais	48 hours	LC_{50}	22,000 μg/L

Source: Verschueren 1983

2.11 Diuron

2.11.1 Fish

Existing toxicity data for Diuron indicate the chemical is moderately to highly toxic to fish (WSDOT 1993). A study looking at the embryo to juvenile stage of freshwater fathead minnow (*Pimephales promelas*) reported a NOEL from an exposure of 33.4 μg/L after a 64-day period (Call *et al*. 1987 *as cited in* Jarvinen and Ankley 1999). A concentration of 4.8 μg/g chemical residue was reported from the fathead minnow body, suggesting that moderate bioconcentration is possible with this chemical.

2.11.2 Aquatic Invertebrates

No recent information was discovered in literature review that identified specific impacts to aquatic invertebrates from Diuron exposure.

2.12 Glyphosate

2.12.1 Fish

Glyphosate is considered practically non-toxic to fish. The reported 96-hour LC_{50} concentrations for technical grade Glyphosate are 120 mg/L for bluegill sunfish and 86 mg/L for rainbow trout (Tu *et al*. 2001c). There is some variability of response by aquatic organisms to different formulations of Glyphosate. For example, Touchdown® 4-LC and Bronco® have low LC_{50}s (<13 mg/L), however Rodeo® has a relatively high LC_{50} (>900 mg/L) and is permitted for use in aquatic systems (Tu et al. 2001c). The LC_{50} of Roundup® for bluegill sunfish and rainbow trout is 6-14 mg/L and 8-26 mg/L, respectively (Tu *et al.* 2001c). Chronic exposures of Glyphosate in fish have resulted in lung damage with concentrations of 5 mg/L and liver damage with concentrations of 10 mg/L over an exposure period of 2 weeks (Tu *et al.* 2001c).

A study looking at the adult life stage of rainbow trout (*Oncorhyncus mykiss*) in an artificial stream reported a NOEL for reproduction from an exposure of 2.0 mg/L after a 30-day period (Folmar *et al*. 1979 *as cited in* Jarvinen and Ankley 1999). A concentration of 80 μg /g chemical residue was reported in a fillet. Additional information on Glyphosate toxicity as found in the herbicide Rodeo® is presented in section 2.22 (Imazapyr).

2.12.2 Aquatic Invertebrates

Glyphosate is considered slightly to practically non-toxic to aquatic invertebrates. The LC_{50} of the chemical Glyphosate alone is 962 mg/L in *Daphnia magna*, but is 25.5 mg/L in the commercial formulation of Roundup® (Tu *et al.* 2001c). Under laboratory conditions emulating an application of Roundup® up to 220 kg/ha did not significantly affect the survival of *Daphnia magna* or its food base of diatoms (Tu *et al.* 2001c). This suggests that under most conditions, formulations of Glyphosate are rapidly dissipated from aquatic environments and does not bioaccumulate in aquatic organisms.

2.12.3 Adjuvant and Inert Ingredient Exposure and Synergism

The surfactant (MONO818®) that is used in commercial formulations of Roundup® is toxic to fish (Tu *et al.* 2001c). The surfactant found in Roundup® is used to aid the breakdown of surface tension on leaf surfaces. The LC_{50} of MONO818® is 2-3 mg/L for sockeye, rainbow, and coho fry (Tu *et al.* 2001c). The surfactant used at times with Rodeo® is estimated to be 100 times more toxic to aquatic invertebrates than Rodeo alone.

2.13 Metsulfuron Methyl

2.13.1 Fish

The 1993 WSDOT EIS reports that Metsulfuron methyl is considered practically non-toxic to fish and aquatic invertebrates. Further examinations of more recent literature did not identify additional aquatic toxicity information.

2.14 Picloram

2.14.1 Fish

Picloram has been considered moderately toxic to fish (WSDOT 1993). There is little evidence of its bioaccumulation. In a static bioassay looking at the response of cutthroat trout to Picloram after a 96 hour exposure, a median threshold limit (TLm) of 3.45 to 8.6 mg/L at 10ºC was reported (Woodward 1976 *as cited in* Verschueren 1983). A separate study, using the same parameters, on the response of Picloram in lake trout reported a TLm of 1.55 to 4.95 mg/L at 10ºC.

Tu *et al.* (2001d) reported a study that exposed rainbow trout, bluegill sunfish, and fathead minnow to Picloram for 96 hours. The resulting LC_{50} concentrations were 19.3 mg/L, 14.5 mg/L, and 55 mg/L, respectively.

2.14.2 Aquatic Invertebrates

Picloram toxicity to aquatic invertebrates has been ranked as practically non-toxic to moderately toxic (WSDOT 1993). Exposing *Daphnia magna* to Picloram for a 48-hour period resulted in a LC_{50} of 68.3 mg/L (Tu *et al.* 2001d). The crustacean *Gammarus lacustris* has a reported 96-hr LC_{50} of 27 mg/L (Sanders 1969, *as cited in* Verschueren 1983).

2.15 Oryzalin

2.15.1 Fish

The 1993 WSDOT EIS reports that Oryzalin is moderately toxic to fish. The reported LC_{50} concentration of Oryzalin in fish after a 96-hour exposure period is 2.88 mg/L in bluegill sunfish, 3.26 mg/L in rainbow trout, and >1.4 mg/L in goldfish fingerlings (BPA 2000c, EXTOXNET 1996c).

2.15.2 Aquatic Invertebrates

Oryzalin was reported as highly toxic to aquatic invertebrates (WSDOT 1993). Recent review of the literature did not identify additional aquatic toxicity information on Oryzalin.

2.16 Sulfometuron Methyl

2.16.1 Fish

Sulfometuron methyl was previously reported as slightly toxic to fish (WSDOT 1993) The Sulfometuron methyl LC_{50} concentrations is reported as >12.5 mg/L for bluegill sunfish and rainbow trout (EXTOXNET 1996d, USDA 1995a). The chemical does not pose an exposure threat to adult aquatic organisms, however the embryo hatch stage of fathead minnow may be sensitive to exposure at concentrations above 0.71 mg/L (EXTOXNET 1996d). There have been reports of fish kills due to exposure to Sulfometuron, but the levels of exposure have not been identified in these reports.

2.16.2 Aquatic Invertebrates

Sulfometuron methyl was previously reported as practically non-toxic to aquatic invertebrates (WSDOT 1993). Recent review of the literature indicates Sulfometuron methyl has a reported LC_{50} concentration of >12.5 mg/L for *Daphnia magna* (water flea) (USDA 1995a). A report by EXTOXNET (1996d) reported a conflicting LC_{50} value of >1,000 mg/L in *Daphnia magna*. Site-specific study would be required to fully ascertain the toxicity of this chemical to aquatic invertebrates.

2.17 Triclopyr

2.17.1 Fish

Triclopyr has been reported to be practically non-toxic to highly toxic to fish (WSDOT 1993). Triclopyr has a LC_{50} concentration of 117 mg/L in rainbow trout and 148 mg/L in bluegill sunfish after a 96-hour exposure period to the parent compound and amine salt (EXTOXNET 1996e, USDA 1995b). The same study for the ester formulation reported a LC_{50} concentration of 0.74 mg/L for rainbow trout and 0.87 mg/L for bluegill sunfish (EXTOXNET 1996e). Potential for bioaccumulation within fish of the chemical is low; the bioconcentration factor for Triclopyr in whole bluegill sunfish is 1.08.

2.17.2 Aquatic Invertebrates

Triclopyr has a LC_{50} concentration of 1,170 mg/L in *Daphnia magna* for the amine salt and 0.74 mg/L (EXTOXNET 1996e). USDA (1995g) reported the LC_{50} concentration in *Daphnia magna* as slightly lower at 1,140 mg/L.

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2.18 Bromoxynil

2.18.1 Fish

The primary active ingredient in Buctril® 2EC is Bromoxynil octanoate (Bayer 2002a). Acute toxicity studies indicate that Bromoxynil octanoate is highly toxic to very highly toxic to freshwater fish species tested. In rainbow trout, an LC_{50} of 0.05 mg/L was reported following exposure to 36.6% Bromoxynil octanoate (Harper 1965 *as cited in* U.S. EPA 1998a). Exposure to 87.3% Bromoxynil octanoate produced an LC_{50} of 0.1 mg/L in rainbow trout (Sousa 1981 *as cited in* U.S. EPA 1998a). An LC_{50} of 0.053 mg/L was reported for bluegill sunfish exposed to 87.3% Bromoxynil octanoate.

Two early life-stage studies using fathead minnow were reported by U.S. EPA. In a study exposing fathead minnow to 97.2% Bromoxynil octanoate, NOEL and LOEL concentrations were 0.018 and 0.039 mg/L, respectively, based on decreased larval growth, survival and embryo hatching success (Soussa, 1991 *as cited in* U.S. EPA 1998a). In the second study, exposure to 63% Bromoxynil octanoate resulted in NOEL and LOEL concentrations of 0.009 amd 0.018 mg/L, respectively, based on decreased larval survival (Suprenant, 1987 *as cited in* U.S. EPA 1998a).

2.18.2 Aquatic Invertebrates

Bromoxynil octanoate was very highly toxic to freshwater invertebrates based on acute toxicity tests. Exposure of the "water flea" *Daphnia magna* to 87.3% Bromoxynil octanoate resulted in an EC_{50} value of 0.096 mg/L (Suprenant, 1981 *as cited in* U.S. EPA 1998a). In *Daphnia pulex* exposed to 36.6% Bromoxynil octanoate, an EC_{50} value of 0.011 mg/L was reported (Harper, 1964 *as cited in* U.S. EPA 1998a).

Two lifecycle tests using *Daphnia magna* were reported by U.S. EPA. Exposure to 97.2% Bromoxynil octanoate resulted in NOEL and LOEL values of 0.025 and 0.059 mg/L, respectively (Putt, 1991 *as cited in* U.S. EPA 1998). Exposure to 60% Bromoxynil octanoate resulted in NOEL and LOEL values of 0.026 and 0.053 mg/L, respectively (Suprenant, 1986 *as cited in* U.S. EPA 1998a).

2.18.3 Adjuvant and Inert Ingredients

The label for Buctril® herbicide indicates that it contains 66.6% inert ingredients consisting of "xylene range/petroleum distillates" (Bayer Crop Science 2002a). The MSDS further states that by weight, Buctril® contains a minimum concentration of the following chemicals: trimethyl benzene (14.8%), xylene (10%), and ethyl benzene (2.3%) (Bayer 2002b). U.S. EPA classifies Ethyl Benzene and

Xylene as inert ingredients that are “Potentially Toxic/High Priority for Testing” (List 2) (U.S. EPA 2004).

2.19 Diflufenzopyr

2.19.1 Fish and Aquatic Invertebrates

U.S. EPA characterizes Diflufenzopyr as slightly toxic to practically non-toxic for both freshwater and marine/estuarine organisms. For freshwater organisms, LC_{50} values ranged from 15 to >135 mg/L. The LC_{50} values for marine/estuarine organisms ranged from 18.9 to >138 mg/L (U.S. EPA 1999). The species tested in these studies was not provided and additional toxicity data were not identified.

2.19.2 Adjuvant and Inert Ingredient Hazards

The label for Overdrive® herbicide indicates that this product contains 23.6% inert ingredients in addition to the active ingredient Diflufenzopyr (BASF 2003). The specific ingredients were not provided on the label or the MSDS.

2.20 Flumioxazin

2.20.1 Fish

Flumioxazin is considered slightly to moderately toxic to fish based on 96-hour acute toxicity studies. In rainbow trout, an LC_{50} of 2.3 mg/L was reported; the LD_{50} for bluegill sunfish was >21 mg/L (U.S. EPA 2001).

2.20.2 Aquatic Invertebrates

Flumioxazin is considered moderately toxic to freshwater invertebrates and moderately to highly toxic to estuarine invertebrates. Acute toxicity studies in *Daphnia pulex* resulted in a 48-hour EC_{50} of 5.5 mg/L (U.S. EPA 2001). Exposure of *Daphnia magna* under the same conditions also resulted in an EC_{50} of 5.5 mg/L. Mysid shrimp exposed in a 96-hour toxicity test resulted in an EC_{50} of 0.23 mg/L. Eastern oyster also exposed in a 96-hour test had an EC_{50} value of 2.8 mg/L based on shell deposition (Valent 2003).

2.20.3 Adjuvant and Inert Ingredients

The MSDS for Payload® herbicide indicates that this product contains 49% “other ingredients” aside from 51% of the active ingredient Flumioxizan. The MSDS further indicates that Payload® contains by weight the following “other ingredients”: kaolin clay (16%) titanium dioxide (<1%) and crystalline silica (<1%) (Valent 2003).

2.21 Fluroxypyr

2.21.1 Fish

Acute toxicity tests evaluated by U.S. EPA indicate that Fluroxypyr is slightly toxic to practically non-toxic to freshwater fish. For bluegill sunfish, a 96-hour LC_{50} >14.3 mg/L was reported. For rainbow trout, 96-hour LC_{50} values ranged from 13.4 mg/L to >100 mg/L (U.S. EPA 1998c).

2.21.2 Aquatic Invertebrates

Results from toxicity testing conducted on *Daphnia magna* indicate that Fluroxypyr is practically non-toxic to this species of invertebrate. The 48-hour EC_{50} for this toxicity test was >100 mg/L. Some estuarine/marine invertebrates were reported to be more sensitive to the toxicity of Fluroxypyr and related compounds. Fluroxypyr acid was highly toxic to eastern oyster with 96-hour LC_{50}/EC_{50} of 0.068 mg/L. Fluroxypyr 1-methyleptyl ester was slightly toxic to the eastern oyster with 96-hour LC_{50}/EC_{50} of 51 mg/L. This compound was practically non-toxic to grass shrimp with a 96-hour LC_{50}/EC_{50} >120 mg/L (U.S. EPA 1998c).

2.21.3 Adjuvant and Inert Ingredients

The MSDS for Vista® herbicide indicates that this product contains 73.8% inert ingredients aside from the active ingredient Fluroxypyr. Although the complete list of these inerts is not included, the following chemicals are listed: 1-methyl-2-pyrrolidinone and petroleum solvent, which includes naphthalene (Dow AgroSciences 2004). U.S. EPA classifies petroleum hydrocarbons as inert ingredients that are “Potentially Toxic/High Priority for Testing” (List 2) although naphthalene is not specifically mentioned in this list (U.S. EPA 2004).

2.22 Imazapyr

2.22.1 Fish

The reported acute toxicity LC_{50} concentration for rainbow trout, bluegill sunfish, and channel catfish is >100 mg/L based on product registrant studies with technical grade Imazapyr using standard 96-hr exposure studies (Mangels and Ritter 2000). Tests were also conducted with the Atlantic silverside to address the potential toxicity of Imazapyr to marine fish. In those tests, the highest concentration tested was 184 mg/L, which yielded no significant toxicity (mortality). As with studies with terrestrial animals, the NOEC was taken as the Highest Dose Tested (HDT), or 100 mg/L for freshwater fish and 184 mg/L for marine fish. On this basis, Imazapyr was considered practically non-toxic to freshwater fish based on toxicity criteria outlined in Table 3-1. A summary report by USDA reported an LC_{50} of <100 mg/L for fish; thus Imazapyr would be characterized as slightly toxic to fish based on the parameters presented in Table 3-1. The species of fish tested was not provided (USDA 1995f).

Imazapyr has not been thoroughly tested for chronic or sub-lethal effects with a wide variety of aquatic organisms, but those few tests conducted are worth summarizing. Early life stage survival tests with rainbow trout and fathead minnow embryos and sac-fry continuously exposed to Imazapyr revealed no effects on hatching or survival at concentrations as high as 92.4 mg a.i./L and 118 mg a.i/L, respectively. Again, these were the highest concentrations tested. A full life cycle test with fathead minnow with concentrations up to 120 mg a.i./L also did not elicit toxicity (Cyanamid 1997).

It is unclear why the product registrant did not pursue testing with higher concentrations to establish the true maximum tolerated dose. Such testing has applications when addressing potential spill scenarios with the highly soluble herbicide. However, recent results by University of Washington researchers help to eliminate this uncertainty (C. Grue personal communication, *as cited in* Fisher *et al*. 2003). Grue and others examined the toxicity of Imazapyr in 96-hr tank tests with juvenile rainbow trout (Table 3-4). As demonstrated in these tests, the concentrations required to achieve 50% mortality are exceedingly high. One purpose of the Grue *et al.* studies was to compare the toxicity of Imazapyr with Rodeo® (Glyphosate); data are summarized for this herbicide as well. As further demonstrated in Table 3-4, the LC_{50} of Glyphosate established in the same trials was approximately two orders of magnitude more toxic than the Arsenal® herbicide.

Table 3-4: 96-hour LC_{50} Values with 0.3 g juvenile rainbow trout exposed to Imazapyr (Arsenal®) or Glyphosate (Rodeo®) tank mixes (Source: C. Grue 2003, personal communication, as cited in Fisher et al. 2003.)

Product Tested	LC_{50} of Concentrate (mg/L)	LC_{50} Expressed as Active Ingredient (mg/L)
Arsenal® Herbicide	77,716 (72,183-72,243)*	22,305 (20,718-20,891)*
Arsenal® Concentrate	43,947 (41,446-46,408)*	23,336 (22,024-22,643)*
Rodeo®	782 (719-845)*	782 (719-845)*

* 95% confidence interval of four replicated trials with geometrically arranged concentrations and a negative control.

Few sub-lethal endpoints other than the early-life-stage and life cycle tests conducted with the standard test species have been fully explored with Imazapyr. One recent study examined the potential for Imazapyr (Arsenal®) and Glyphosate (Rodeo®) to elicit micronuclei in the African cichlid fish (*Tilapia rendalli*) injected intraperitoneally with the herbicides (Grisolia 2002). Micronuclei, reflected as chromosomal abnormalities in blood smears, have been proposed as a reliable indicator of environmental mutagenesis in aquatic and terrestrial animals, and have been evaluated in a variety of mollusc, fish and amphibians as an indicator of potential mutagenicity (Al-Sabti and Metcalfe 1995, Vernier *et al.* 1997). However, the significance of elevated micronuclei frequency at the population level has not been fully determined. In the Grisolia (2002) study, significantly elevated numbers of micronuceil were observed following Imazapyr exposure, but only at 80 mg/kg-bw, the maximum tolerated dose (MTD). Evidence of sub-lethal effects at the MTD is not considered a valid indicator of sub-lethal toxicity, as the fish are exhibiting overt cytotoxicity (cell death) signs. Chromosomal aberrations such as micronuclei are common during cell death; their significance to mutagenicity studies is relevant when occurring as a sub-lethal toxicological response to chemical exposure doses below those which cause cell death.

2.22.2 Aquatic Invertebrates

Imazapyr would be considered slightly toxic to practically non-toxic to invertebrates based on the results from a range of invertebrate species. The reported acute toxicity LC_{50} concentration for the water flea *Daphnia magna* is >100 mg/L (Tu *et al*. 2001g, BPA 2000). One study where Arsenal was applied with a surfactant (not defined) with the *Daphnia* yielded a 48-hr LC_{50} of 350 mg-Arsenal/L (79.1 mg a.i. Imazapyr/L) and a NOEC of 180 mg-Arsenal/L (40.7 mg a.i./L). Other product registrant studies where *Daphnia* was exposed to an Imazapyr formulation (~50%) lacking the surfactant produced a 48-hour EC_{50} concentration of 373 mg a.i./L (Cyanamid 1997). The results of these two studies highlight the potential effect of surfactant on aquatic toxicity, and the authors concluded "components of the Arsenal® formulation, other than a surfactant, do not influence the toxicity of Imazapyr to aquatic organisms." Kintner and Forbis (1983) also reported 24 and 48-hour LC_{50} concentrations of greater than 100 mg/L (the HDT), in static tests conducted with newly-hatched *Daphnia* (less than 24 hours old). Chronic studies have also been conducted with the water flea (Manning 1989). In that study, no adverse effects on survival, reproduction or growth of 1st generation *Daphnia* were recorded after 7, 14 and 21-days of exposure at concentrations up to 97.1 mg/L, the HDT. Per FIFRA registration requirements, the NOEC was considered to be the HDT (97.1 mg/L), and the maximum allowable toxicant concentration (MATC) was considered to be >97.1 mg/L. A summary report by USDA reported an LC_{50} of <100 mg/L for water flea (USDA 1995f).

Testing with other invertebrate species that exhibit alternative life cycles has been limited to growth studies with the Eastern oyster (*Crassostrea virginica*), and survival of pink shrimp. Although these species are not native to coastal Washington, they do provide reasonable surrogates for the Pacific oyster (*Crasostrea gigas*) and burrowing shrimp (*Neotripia spp*.) that are common and commercially valuable species to areas where Spartina has become established. In these product registrant tests, the EC_{50} for growth inhibition was established at a concentration >132 mg-Imazapyr/L, with the NOEC set at this concentration—the HDT. The pink shrimp survival LC_{50} was >189 mg-Imazapyr/L, and the NOEC was again set at this HDT (Mangels and Ritter 2000).

2.22.3 Adjuvant and Inert Ingredients

The labels for Habitat® and Arsenal® herbicides indicate that these two herbicides contain 71.3% inert ingredients in addition to 28.7% of the active ingredient Imazapyr (BASF 2000, BASF 2004). The MSDS for Arsenal® indicates that the formulation by weight contains 46.9% inert ingredients and 54.1% Imazapyr (BASF 2000c). The reason for the discrepancy between the label and MSDS is not known. The specific inert ingredients were not provided by the manufacturer; however, Grisolia, *et al*. (2004) indicate that the surfactant nonylphenol ethoxylate may be used with Imazapyr. Information on the toxicity of the surfactant nonylphenol ethoxylate to fish and aquatic invertebrates was not available among the reference sources searched (Section 1.2). Elsewhere, acetic acid has been listed as an inert ingredient of Arsenal® (NCAP 2003 see Section 3.20).

2.23 Isoxaben

2.23.1 Fish and Aquatic Invertebrates

It was not possible to evaluate the toxicity of Isoxaben or aquatic invertebrates because adequate data were not identified. The bioconcentration potential of Isoxaben is reportedly low with a bioconcentration factor (BCF) <100 (Dow 2003).

2.23.2 Adjuvant and Inert Ingredients

The label for Gallery* 75® Dry Flowable herbicide indicates that it contains 25% “other ingredients” including Kaolin and crystalline silica (in Kaolin) (Dow AgroSciences 2003). Information on these particular compounds was not identified among the reference sources searched (See Section 1.2); therefore, toxicity to fish and aquatic invertebrates was not evaluated for this herbicide.

2.24 Norflurazon

2.24.1 Fish

Norflurazon was moderately to slightly toxic to freshwater fish based on 96-hour acute toxicity studies using 98.6% active ingredient. For rainbow trout and bluegill sunfish, LD_{50} values were 8.1 and 16.3 mg/L, respectively (Stoll *et al* 1981 *as cited in* U.S. EPA 1996). Early life-stage toxicity tests resulted in NOEC and LOEC values for survival and growth of larvae in rainbow trout of 0.77 and 1.5 mg/L, respectively. For fathead minnow, a chronic toxicity study reported NOEC and LOEC concentrations of 1.1 and 2.1 mg/L for weight and length development (EG&G Bionomics 1982 *as cited in* U.S. EPA 1996).

2.24.2 Aquatic Invertebrates

U.S. EPA reported that Norflurazon is slightly toxic to freshwater invertebrates based on a NOEC of 15 mg/L in *Daphnia magna* (Vilkas 1980 *as cited in* U.S. EPA 1996). In estuarine and marine invertebrates, Norflurazon was classified as slightly to moderately toxic based on acute toxicity studies. For mysid (*Mysidopsi bahia*), an LC_{50} of 5.53 mg/L was reported following exposure to 99.4% active ingredient (Reed *et al.* 1991 *as cited in* U.S. EPA 1996). In toxicity tests with the Eastern oyster, an EC_{50} of 3.8 mg/L and a NOEC of 1.2 mg/L were reported based on shell deposition following exposure to 98.6% active ingredient (Graves and Swigert, 1993 *as cited in* U.S. EPA 1996). A NOEL >10 mg/L was reported for Atlantic oyster embryo larvae exposed to 98.8% active ingredient (Bently 1973 *as cited in* U.S. EPA 1996).

In a life cycle toxicity test using *Daphnia magna* with exposure to 96.6% active ingredient, NOEC and LOEC values of 1.0 and 2.6, respectively, were reported based on percent survival and offspring production (EG&G Bionomics 1983 *as cited in* EPA 1996).

2.24.3 Adjuvant and Inert Ingredients

The label for Predict® herbicide indicates that this product contains 21.4% “other ingredients” aside from the active ingredient Norflurazon. The specific “other” ingredients were not provided by the manufacturer.

2.25 Oxadiazon

2.25.1 Fish

Oxadiazon is moderately to highly toxic to fish based on acute toxicity tests. In studies evaluated by U.S. EPA, an LC_{50} of 0.88 mg/L was reported for bluegill and rainbow trout. For sheepshead minnow, a marine/estuarine fish, the LC_{50} was reported as 1.5 mg/L (U.S. EPA 2003). Elsewhere, the LC_{50} for Oxadiazon was reportedly >2 mg/L for all freshwater species tested (Weed Science Society of America, 1983 *as cited in* Cornell University, 2001).

A chronic test using rainbow trout resulted in an unspecified endpoint at 0.0008 mg/L. For sheepshead minnow, results from a chronic test were reported as 0.0037 mg/L based on an unspecified endpoint (U.S. EPA 2003)

2.25.2 Aquatic Invertebrates

Oxadiazon is moderately toxic to aquatic invertebrates based on an LC_{50} of 2.2 mg/L reported for *Daphnid* (water flea). An LC_{50} of 0.27 mg/L was reported in mysid shrimp, suggesting that Oxadiazon is highly toxic to estuarine invertebrates (U.S. EPA 2003).

2.25.3 Adjuvant and Inert Ingredients

The label for Ronstar 50 WSP® herbicide indicates that this product contains 50% “inert ingredients” aside from the active ingredient Oxadiazon (Bayer 2004). The specific inert ingredients were not provided by the manufacturer.

2.26 Pendimethalin

2.26.1 Fish

Pendimethalin is moderately to highly toxic to fish based on acute toxicity studies evaluated by U.S. EPA using 93.2% (technical grade) active ingredient. The LC_{50} values for rainbow trout, bluegill sunfish and channel catfish were 0.138, 0.199, and 0.418, respectively (Sleight 1973 *as cited in* U.S. EPA 2003). In studies using typical formulated product with 45% active ingredient, LC_{50} values were 0.52, 0.92 and 1.9 mg/L respectively for rainbow trout, bluegill sunfish (Bentley1974 *as cited in* U.S. EPA 2003), and catfish (Sousa 1983 *as cited in* U.S. EPA 2003).

A chronic life-cycle study involving fathead minnow exposed to 98.3% a.i. resulted in a NOEC of 0.0063 mg/L and a LOEC of 0.0098 mg/L for reduced egg production (EG&G Bionomics 1975 *as cited in* U.S. EPA 2003).

2.26.2 Aquatic Invertebrates

Technical grade Pendimethalin was found to be highly toxic to freshwater invertebrates based on acute toxicity LC_{50}/EC_{50} values of 0.28 and 1.0 mg/L, respectively, for *Daphnia magna* and crayfish (*Procambarus simulans*) (EG&G Bionomics, 1976 and ABC Inc, 1980 *as cited in* U.S. EPA 2003). A study that exposed *Daphnia magna* to formulated Pendimethalin (45.6%) resulted in a LC_{50}/EC_{50} value of 5.1 mg/L (Forbis 1985 *as cited in* U.S. EPA 2003).

2.26.3 Adjuvant and Inert Ingredients

The two Pendimethalin-containing herbicide formulations suggested for use by WSDOT contain varying degrees of inert ingredients. Pendulum® WDG contains 40% inert ingredients that are not specified by the manufacturer (BASF 2001); Pendulum 3.3 EC contains 62.6% inert ingredients that include petroleum distillates (BASF 2004). U.S. EPA classifies petroleum hydrocarbons as inert ingredients that are "Potentially Toxic/High Priority for Testing" (List 2) (U.S. EPA 2004).

2.27 Pyraflufen

2.27.1 Fish

Findings from acute toxicity tests ranged widely from highly toxic to practically non-toxic for 96-hour acute toxicity studies reviewed by the European Commission (EC). The LC_{50} for rainbow trout ranged from >0.1 to <60 mg/L, while for bluegill sunfish an LC_{50} >100 mg/L was reported. In a longer term study, a NOEC of 10 mg/L was reported for fathead minnow (EC 2002).

2.27.2 Aquatic Invertebrates

Findings from acute toxicity tests ranged widely from highly toxic to practically non-toxic for 48-hour acute toxicity studies of *Daphnia magna*. The three separate studies summarized by European Commission (EC) resulted in EC_{50} values of >0.1, >120 and >15 mg/L (EC 2002).

2.27.3 Adjuvant and Inert Ingredients

The label for Edict® IVM herbicide indicates that this product contains 97.5% "other ingredients" aside from the active ingredient Pyraflufen (Nichino America 2004). The specific inert ingredients used in this product were not provided by the manufacturer.

2.28 Sulfentrazone

2.28.1 Fish

Acute toxicity tests reviewed by U.S. EPA indicate that Sulfentrazone is practically non-toxic to slightly toxic in fish. The LC_{50} for rainbow trout was >120 mg/L; for bluegill sunfish, an LC_{50} of 94 mg/L was reported. Developmental toxicity studies suggest Sulfentrazone can adversely impact survival and growth of young fish at concentrations down to 5.9 mg/L (U.S. EPA 1997).

2.28.2 Aquatic Invertebrates

U.S. EPA reported that Sulfentrazone is slightly toxic to aquatic invertebrates on an acute basis, although specific acute test results were not provided. Based on a chronic toxicity test in daphnids ("water flea"), it was reported that exposure to 0.51 mg/L adversely impacted survival of young (U.S. EPA 1997).

2.28.3 Adjuvant and Inert Ingredients

The label for Portfolio® herbicide indicates that this product contains 25% inert ingredients aside from the active ingredient Sulfentrazone (Wilbur-Ellis). The specific inert ingredients were not provided by the manufacturer.

2.29 Tebuthiuron

2.29.1 Fish

Tebuthiuron toxicity ranges from practically non-toxic to slightly toxic to fish based on 96-hour acute toxicity tests evaluated by U.S. EPA. Reported LC_{50} values for technical grade Tebuthiuron (98%) were 143 mg/L and 106 mg/L for rainbow trout and bluegill sunfish, respectively. In studies using formulated product (both 80% and 20%), LC_{50} values for fathead minnow were >180 mg/L (U.S. EPA 1994). Acute LC_{50} ranges of 87 – 144 mg/L for rainbow trout and 87 – 112 mg/L for bluegill sunfish were reported elsewhere (EXTOXNET 1996f). For acute toxicity 96-hour tests using the fathead minnow, an LC_{50} value of 180 mg/L was reported for both 80% wettable powder (WP) and 20% pelleted/tabletted (P/T) formulations (U.S. EPA 1994).

In early life-stage studies of fish exposed to 98% a.i., survival and growth were impaired in rainbow trout at levels >26 mg/L but <52 mg/L, while in fathead minnows, growth was impaired at levels >9.3 but <18mg/L. Survival in the fathead minnow was unaffected at levels up to 76 mg/L (U.S. EPA 1994).

2.29.2 Aquatic Invertebrates

Tebuthiuron is considered practically non-toxic to aquatic invertebrates based on an LC_{50} of 297 mg/L reported in *Daphnia magna* exposed to 99.2% active ingredient. In marine/estuarine organisms, LC_{50} values were >180 but <320 mg/L for Eastern oyster and 62 mg/L for pink shrimp. Life cycle tests conducted on *Daphnia magna* indicate a significant reduction in growth and fecundity from Tebuthiuron at levels >21.8 but <44 mg/L (U.S. EPA 1994).

2.29.3 Adjuvant and Inert Ingredients

The label for Spike® 80 DF herbicide indicates that this product contains 20% inert ingredients aside from the active ingredient Tebuthiuron (Dow AgroSciences 1999). Specific inert ingredients used in this product were not provided by the manufacturer.

2.30 Hazards from Adjuvant and Inert Ingredients Toxicity to Ecological Receptors

Adjuvants are carriers mixed with herbicides that increase the binding and/or uptake of the herbicide into target plants. Typical adjuvants include surfactants and crop oils that are mixed with the herbicide prior to application. Inert ingredients are components within the patented herbicide product formulations that are reported to have no herbicidal activity. Current FIFRA regulations do not require manufacturers to reveal the surfactant formulations, as FIFRA regulates the active ingredients only. Similarly, herbicide toxicity studies conducted under FIFRA are required to evaluate the active ingredient of the product formulation only, and not the toxicity of the "inert ingredients" or the surfactants that may be used to facilitate plant adsorption and uptake of the herbicide. For some ecological receptors, particularly aquatic receptors, the choice of which surfactant to use to administer the herbicide can have substantial ecological relevance, as the few tests conducted with surfactants have shown higher toxicity than the herbicide. Similarly, in environments where a variety of herbicides and/or pesticides may be used, the potential for chemical interactions of inert ingredients should also be understood to minimize risks. This section of the hazard assessment therefore attempts to summarize the existing information on the toxicity inherent to the inert ingredients and surfactants that could be used in the application of Imazapyr to control *Spartina*.

2.30.1 Inert Ingredients

Among the inert ingredients listed in the herbicides evaluated in 2005 are petroleum distillates in Buctril® 2EC (Bromoxynil), Vista® (Fluroxypyr), and Pendulum (Pendimethalin). Petroleum distillates are listed by U.S. EPA (2004) as "Potentially Toxic/High Priority for Testing" (List 2). Among the specific inert ingredients listed as part of the Buctril® 2EC MSDS (Bayer 2002b) are xylene and ethyl benzene (both List 2 inert ingredients) and trimethyl benzene. Although U.S.EPA classifies these petroleum hydrocarbons as inert ingredients based on their use in pesticides, this class of compound is known to exhibit a wide variety of potential toxicological effects including reproductive, developmental and neurological effects and potential carcinogenicity (Merck 1989). Other inert ingredients listed among the herbicides assessed in 2005 include kaolin clay (16%), titanium dioxide (<1%), and crystaline silica (<1%) in Payload® (Flumioxizan), kaolin and crystalline silica in Gallery® 75 DF and 1-methyl-2-pyrrolidinone in Vista® (Fluroxypyr). Kaolinite clay is classified by U.S. EPA (2004) as "Generally regarded as safe" (List 4a); however, more detailed information on these inert ingredients was not available through the literature searched (see Section 1.2). Two of the inert ingredients in Arsenal® (Imazapyr) are listed as glacial acetic acid and water (NCAP 2003). Acetic acid is a common inert ingredient which is classified as a List 4a inert ingredient by U.S. EPA (2004). The toxicity of acetic acid is tabulated below (Table 3-5), as summarized by Merck (1989) and Verschueren (1983). Acetic acid is also a component of LI 700, a common non-ionic surfactant with potential use with Imazapyr.

Table 3-5: Acetic Acid Toxicity to Ecological Receptors (source Fisher *et al*. 2003)

Test species	Class of organism	Toxicity test	Toxicity end point	Value	Unit
Brine shrimp	Arthropoda	TLm*	NA	32-47	mg/l
Grammarus pulex	Arthropoda	TLm*	NA	6	mg/l
Limnea ovata	Mollusca	NA	Perturbation level	15	mg/l
Bluegill	Fish	TLm* (24, 96-hr--respectively)	NA	100-1000, 75	mg/l
Mosquito fish	Fish	TLm (24-96 hr)	NA	251	mg/l
Fathead minnow	Fish	LC_{50} (1, 24, 48, 72, 96-hr--respectively)	Death	175, 106, 106, 79, 79)	mg/l

*median tolerance limit

2.30.2 Surfactants

Surfactants are used to reduce the surface tension of water, enabling a "bridge" to form between two chemicals or media that would not normally mix (*e.g.*, oil and water). When used with herbicides, they are intended to maximize the amount of spray solution that sticks to the leaf surface, and hence increase uptake. Surfactants commonly used to promote adsorption and uptake are generally of two classes: non-ionic nonylphenol alcohols and/or fatty acids, and crop-oil based concentrates (Fisher *et al*. 2003). Studies evaluating the efficacy of herbicides with various surfactants have revealed few differences in the efficacy of the herbicides to target plants based on the surfactant (Patten 2002). All surfactants tested with one herbicide (Imazapyr) provided effective control, but R-11, the approved surfactant for use with another herbicide (Glyphosate), was not tested with Imazapyr, making a direct comparison difficult (Table 3-6). However, the author states that "application made with short dry time might better distinguish surfactant effects than did these trials, all of which had ample dry time".

Table 3-6: Effect of surfactant applied in September 1999 and 2000 on the efficacy of Imazapyr for smooth cordgrass control in Willapa Bay, WA (source Patten, 2002.)

Herbicide	Rate (kg/ha)	Surfactant	Percent (v/v)	Percent control 13 months after treatment		
				Site 1	Site 2	Site 3
Imazapyr	1.68	Agri-Dex	1.0	99	85	96
	1.68	Agri-Dex	2.0		96	
	1.68	Hasten	1.0	100	83	94
	1.68	Kinetic	0.5		89	
	1.68	Dyn-Amic	1.0		96	
	1.68	Syl-Tac	1.0		92	
Glyphosate	8.4	R11	1.0		69	85
Untreated	na	na	Na	0	0	0

Although there appears to be little difference amongst surfactants in their potentiation of herbicide efficacy, their inherent chemical properties can have a range of environmental issues that are independent of the herbicide formulation they may be applied with. For this reason, it is prudent to examine their properties and toxicity independently. Table 3-7 summarizes descriptions of surfactant environmental fate, chemistry and toxicity as provided from previously published materials (Fisher *et* *al.* 2003).   In brief, the acute toxicity of alkylphenol ethoxylate surfactants like R-11$_{tm}$ and X-77 $_{tm}$ to fish and other aquatic species has been reported in the range of 4 to 12 mg/L. Acidifying agents like LI-700, and crop-oil based surfactants like Hasten ® and Agri-Dex® exhibit lower toxicity. On the basis of U.S. EPA aquatic toxicity criteria, all the surfactants used would be considered practically non-toxic (LI700®, Hasten®and Agri-Dex®) to moderately toxic (R-11, X-77). In mammals, all of the surfactants can cause irritation to skin and ocular tissue at high doses, and receive ratings of moderate (scores of 4 to 6 on an 8 pt scale) irritation in mammals (Table 3-8). By oral administration, the limited testing done with the surfactants in mammals indicates they would classify as "practically non-toxic."

Table 3-7. Chemistry and Fate of Surfactants Potentially Used With WSDOT Herbicides

Surfactant	**Known Ingredients* & Surfactant Class**	**Chemical Properties**	**Degradation Rate and Pathway**	**General Toxicity Rating***
R-11® (surface activator), Wilbur-Ellis Co.	Isopropyl (butyl) alcohol 20%, nonionic surfactants 80% (octyl phenoxy polyethoxy), silicone. Class: Nonionic alkylphenol ethoxylate	Soluble in lipid & water, Flammable, Spec. Gravity = 1.0	Slowly biodegraded by progressive shortening of ethoxylate chain; intermediate breakdown products of polytheylene glycol (anti-freeze) and short-chain ethoxylates.	Mammals: practically non-toxic orally, mild skin irritation possible Fish and other aquatic biota: moderately toxic
LI-700 ® (penetrating surfactant), Loveland Industries, Inc.	Phosphatidylcholine (lecithin) at 800 g/L, propionic acid, and alkylphenyl hydroxypolyoxyethylene Class: Acidifying agent	Soluble in lipid & water, Not Flammable Spec. Gravity = 1.03	Biodegradation presumed rapid due to natural lecithin ingredients.	Mammals: practically non-toxic orally, but causes skin irritation Fish and other aquatic biota: moderately toxic
X-77® (spreader activator), Valent Corp.	Alkylarylpoly (oxyethylene), glycols, free fatty acids, isopropyl alcohol. Class: Nonionic alkylphenol ethoxylate	Soluble in lipid &water, Flammable	Slowly biodegraded by progressive shortening of ethoxylate chain; intermediate breakdown products of polytheylene glycol (anti-freeze) and short-chain ethoxylates.	Mammals: practically non-toxic orally Fish and other aquatic biota: moderately toxic
HASTEN ®	Proprietary: fatty acids from seed oils esterified with alcohol Class: oil based surfactant	Non-ionic, dispersible in water as micelles, but unknown solubility. Sp. Gravity = 0.9	Biodegradation presumed rapid, but no formal studies conducted of which we are aware.	Mammals: practically non-toxic through oral routes Fish and other aquatic biota: slightly toxic

Surfactant	Known Ingredients* & Surfactant Class	Chemical Properties	Degradation Rate and Pathway	General Toxicity Rating*
AGRI-DEX ®	Proprietary: heavy range paraffin-based petroleum oil with polyol fatty acid esters and polyethoxylyated derivatives Class: oil based surfactant	Dispersible in water (forms micelles), moderate flammability,	Biodegradation presumed rapid, but no formal studies conducted of which we are aware	Mammals: practically non-toxic through oral ingestion, mild skin and eye irritant, Fish and other aquatic biota: practically non-toxic

*See Table 3-1 and Chapter 2 Table 2-1 for toxicity classification schemes

Past studies with Glyphosate have shown that the toxicity of surfactants is generally greater than the toxicity of the herbicide formulation or active ingredient alone. For example, studies with Rodeo® formerly discussed in the original EIS relate how the toxicity of the Rodeo® formulation was 1,100 mg/L without surfactant, and 680 mg/L with the mixture containing 0.4 percent X-77 (Mitchel *et al*. 1987). A similar relationship has been observed with aquatic invertebrates with Rodeo® (Henry 1992). Recent studies with both Imazapyr (Arsenal®) and Glyphosate (Rodeo®) examined the inherent toxicity of the surfactants also, both with and without the herbicides (Smith *et al*. 2002, unpublished data). As demonstrated in Table 3-9, the toxicity of the seed and crop-oil based surfactants Hasten and Agri-Dex to rainbow trout was two to three orders of magnitude lower, respectively, than R-11 in this study. When surfactant was mixed with herbicide, the toxicity of the surfactant was reduced and the toxicity of the herbicide was increased. These studies reveal that the toxicity associated with herbicide/surfactant mixtures is not additive, and is generally associated with the surfactant. Of the surfactants examined in detail, the order of toxicity, from lowest to highest, would appear to be as follows: Agri-Dex, Hasten, LI700, X-77 and R-11. It is noteworthy that only R-11, the surfactant that appears most toxic from the recent tests, is approved for use with Glyphosate in the estuarine environment where herbicide treatment of *Spartina* is conducted.

Table 3-8. Toxicity of Surfactants With and Without Herbicide

(Sources: Smith et al. 2002, Mitchell et al. 1987, Fisher et al. 2003.)

Chemical Tested	Mammalian Toxicity LD_{50} (mg/L)	Aquatic Toxicity (mg/L)
R-11 surfactant	5,840 oral, 13000 dermal (rabbit)	6.0, rainbow trout 96-hr LC_{50*} 4.2, bluegill sunfish 96-hr LC_{50}
LI-700 surfactant	>5,000 oral, 5,000 dermal (rat)	17, rainbow trout 96-hr LC_{50*} 22, rainbow trout 24-hr LC_{50*} 210, bluegill sunfish 96-hr LC_{50} 190, daphnia 48-hr LC_{50}
Hasten® surfactant	No Data	74, rainbow trout 96-hr LC_{50*} 98, rainbow trout 24-hr LC_{50*}
Agri-Dex® surfactant	>5,010 oral (rat), > 2,020 dermal (rabbit)	271, rainbow trout 96-hr LC_{50*} 386, rainbow trout 24-hr LC_{50*}
X-77 surfactant	> 5,000 oral (rat), > 5,000 dermal (rabbit)	4.2, rainbow trout 96-hr LC_{50} 4.3, bluegill sunfish 96-hr LC_{50} 2, water flea (daphnia) 48-hr LC_{50}
Rodeo® (as Glyphosate)	3,800 oral, 5,000 dermal (rabbit)	580, rainbow trout 96-hr LC_{50} 545, water flea (daphnia) 48-hr LC_{50}
Rodeo® + X-77	No Data	130, rainbow trout 96-hr LC_{50} 130, water flea (daphnia) 48-hr LC_{50}
Rodeo® + R-11	No Data	5.4, mg/L rainbow trout 96-hr LC_{50*}

Chemical Tested	Mammalian Toxicity LD_{50} (mg/L)	Aquatic Toxicity (mg/L)
Rodeo® + LI700	No Data	23, mg/L rainbow trout 96-hr LC_{50*}
Arsenal® + Hasten®	No Data	113, mg/L rainbow trout 96-hr LC_{50}
Arsena®l + Agri-dex®	No Data	479, mg/L rainbow trout 96-hr LC_{50*}

*Unpublished data from Smith *et al.* (submitted to Bull. Env. Of Contam. And Tox.). Data represents mean of 4 trials, upper 95% confidence limit within 5 to 20% of mean over all herbicide trials (not shown)

The non-ionic alkylphenol derived surfactants may pose additional hazards beyond the evidence provided in acute toxicity tests. The alkylphenols and octyl phenol ethoxylates belong to a broader class of chemicals known as the "nonylphenols." It has been estimated that approximately 80 percent of the alkyl phenol ethoxylates are nonyl phenol ethoxylates and the other 20 percent are octyl phenol ethoxylates (Cox 1998). Because these compounds are not part of the herbicide formulation, their exact formulations are patent protected and are not reportable under FIFRA. However, the U.S. EPA considers the nonylphenols as an "inert of toxicological concern." Nonylphenol ethoxylates degrade to nonyl phenol and related compounds that can be somewhat persistent in the environment. Sublethal effects at exposure concentrations below acutely toxic levels include impaired swimming activity, altered breathing rate, and reduced heart rate in fish at 0.5 mg/L, and inhibited siphon retraction, byssal thread formation and reduced burrowing activity in sessile shellfish at concentrations greater than 1 mg/L (WSDA 1993a). Lethal effects as reported in the literature are summarized in Table 3-9. The intermediate breakdown products of these surfactants can include both linear and branched chain alkylphenols, which may also have inherent toxicity. Some of these products have been shown to elicit weak estrogenic effects when administered at high doses to laboratory animals. Determining the actual quantity of alkylphenols in each surfactant formulation, and their potential environmental concentrations and risks, is not entirely possible because the proportions in each surfactant formulation are not known.

Table 3-9: Acute toxicity of nonylphenol to aquatic biota.

Test species	Class of organism	Toxicity test	Toxicity end point	Value	Units
Mytilus edulis[1]	Mussel	Bioconcentration Factor	NA	10	Wet weight
Caenorhabditis elegons[2]	Nematode	LC_{50} (24 hr)	Death	7.2	mg/l
Mysidopsis bahia[2]	Mysid	LC_{50} (96 HR)	Death	43	mg/l
Fathead minnow[2]	Fish	LC_{50} (96 HR)	Death	135	mg/l
Gadus morhua[2]	Fish	LC_{50} (96 HR)	Death	3000	mg/l

A group of inert ingredients, polyethoxylated tallow amines, used in some commercial formulations of Clopyralid are acutely toxic to fish (Cox 1998). Adjuvants in some formulations, such as cyclohexanone, triisopropanolamine, and triethylamine, may cause toxicity to terrestrial animals but their toxicity to aquatic animals has not been fully explored. The relative risks from these ingredients cannot be fully ascertained, as the specific ratio of these adjuvants within the formulations has not been reported.

Information regarding aquatic environment toxicity to adjuvant and inert ingredients of Clopyralid/2,4-D commercial formulations was not reported in the compendium of literature we examined. Toxic hazards associated with the inert and adjuvant ingredients of Clopyralid could also be considered associated with the Clopryalid/2,4-D mixture, but again, the absence of information on the ratio of these adjuvants to the active ingredient precludes a full characterization of the risks these ingredients may pose.

3.0 Aquatic Risk Assessment

3.1 Exposure Assessment Methodology

Exposure assessment involves the estimation of the amount of a chemical that an individual or a population may potentially encounter, and considers the extent, frequency, and duration of such exposures. In the aquatic environment, herbicides may be transported and accumulated in various media (sediment, water, plants, aquatic invertebrates, and fish) and/or degraded by a variety of biological, physical, and chemical mechanisms (WSDOT 1993a). It was not possible to ascertain the actual aquatic exposures of all herbicides used by WSDOT in all aquatic environments around the state because adequate empirical data simply do not exist. Thus, exposure was estimated qualitatively for each herbicide based upon the fate and transport of the chemical and WSDOT's stated application rates.

The exposure of aquatic organisms was assumed to be primarily from surface runoff, which may occur when storm events or irrigation closely follows the timing of the herbicide application by WSDOT or one of its contractors. However, aerial drift will also contribute to herbicide concentrations that reach surface waters that support aquatic life. The Washington State Department of Agriculture (WSDA) does not maintain records of use or sales of herbicides for either agricultural or private use, so it was neither practical nor possible to determine to what extent exposure from these other sources might occur.

For the exposure assessment aspect of this study, the following assumptions were made:

- All of the herbicides are used throughout all of the physiographic provinces. This approach maintains a conservative approach to the analysis, but could be refined through further analysis.
- In order to estimate the exposure of aquatic organisms to those chemicals applied to WSDOT rights-of-way for vegetation management, four parameters were considered:

  - Annual rainfall,
  - Road density,
  - Maximum application rate of the active ingredient as reported by WSDOT, and
  - The soil half-life of each chemical.

Rainfall ratings of each parameter were developed in the original EIS, and are reproduced below in Table 3-10.

Table 3-10. Rating Scale for Relative Exposure to Herbicides in Aquatic Environments of Washington State.

Parameter	Rating		
	1	**2**	**3**
Annual Rainfall	0 to 50 inches/year	50 to 90 inches/year	>90 inches/year
Road Density	Low	Medium	High
Maximum Application	0.08 to <1.00 lbs/acre	1.00- <10.00 lbs/acre	>10.00 lbs/acre
Soil half life	0 to 3 months	4 to 6 months	>6 months

Source: WSDOT 1993

The relative exposure rating was calculated as:

$$1)\quad \frac{\text{Annual Rainfall} + \text{Road Density} + \text{Persistence in Soil} + \text{Application Rate}}{4}$$

In order to maintain the same rating schedule (i.e., 1 = low, 2 = moderate, and 3 = high) for relative exposure as for the parameters used to estimate it, the highest sum obtained by adding the maximum relative ratings of rainfall, road density, application rate, and soil half life (i.e., 3 + 3 + 3 + 3 = 12) was divided by a factor of 4. In this manner a relative exposure rating of 3 would indicate a high potential for exposure of aquatic organisms to the selected herbicides. Relative road density and rainfall by physiographic province are provided in Table 3-10 and 3-11 at the end of the chapter. Relative application rates and persistence (soil half life) of each herbicide are provided in Table 3-12. Application rates used by WSDOT are also discussed in Chapter 1 of the EIS.

3.2 Relative Risk Characterization

This section discusses the estimated risks to aquatic wildlife receptors (that is, the exposed organism) that might be inadvertently exposed to WSDOT-applied herbicides as a result of the WSDOT Vegetation Management program. Risks to aquatic ecological receptors from exposure to a potentially hazardous chemical are determined by three factors: duration of exposure, the concentration or dose of the chemical, and the potency of the chemical. Without empirical data for exposure, calculating the probability of a toxicological event to aquatic receptors from exposure is not possible. Thus, a formal probabilistic risk *assessment* is not possible with the current data available, and risks must be *characterized* based on estimates of exposure and effect.

For this assessment, the relative hazard of individual herbicides to aquatic receptors was based initially on acute LD_{50} toxicity criteria concentrations listed in Table 3-1, and then on expansion of the research cited. Because a range of toxicities is often recorded for aquatic species, even for the same species, we took the mean of the reported toxicity ranking for each chemical if a range was supported by the literature, consistent with the original EIS (WSDOT 1993). If the ranking spanned only two adjacent toxicity rankings, the higher toxicity ranking was used in the calculation of relative risk. The description of the range of toxicity rankings (*i.e.*, 1 to 5) is provided in Table 3-16. The toxicity rating for each herbicide was then multiplied by the exposure rating of 1 to 3 for each physiographic region (summarized in Table 3-16) and the product was then divided by a factor of three:

$$2)\quad \text{Relative Risk} = \frac{Exposure \times Toxicity}{3}$$

The divisor factor of three was used in order to maintain the same rating scale as was used for estimating relative toxicity (*i.e.*, 1 to 5). Thus, the highest product obtained (15) was divided by a factor that would result in the highest risk factor corresponding to a value of five. Relative risk values are rounded to the nearest whole number except for values below 0.5 which were rounded up to 1.

Estimates of chemical persistence, exposure, relative risk, and herbicide toxicity to aquatic receptors are summarized in Tables 3-12 to 3-16 at the end of the chapter. Additional text of relevance to the exposure and risk characterization for each herbicide used is summarized below. Because insufficient toxicity or environmental fate data were available for the adjuvants utilized by WSDOT, no exposure or risk characterizations were performed for these substances.

3.3 2,4-D

3.3.1 Exposure Assessment

2,4-D released to surface water will biodegrade relative to: 1) amount of nutrients, 2) temperature, 3) available oxygen, and 4) past history of contamination (Spectrum 2003). Typical half-lives in water range from 10 to >50 days. In still water, 2,4-D has been detected after 6 months of application (USDA 1995c).

Exposure for fish and aquatic invertebrates involves direct contact to surface water that may contain the herbicide due to runoff after application to soils. Bioaccumulation of 2,4-D in aquatic organisms is low; therefore, the potential of exposure through ingestion of exposed aquatic invertebrates, plankton or other food sources to fish and other aquatic life is reduced (WSDOT 1993). As demonstrated in Table 3-13, relative exposure of aquatic organisms to 2,4-D in the physiographic provinces of the state are in the low to moderate range, with the Puget Trough potentially having the highest exposure conditions.

3.3.2 Risk Characterization

3.3.2.1 Fish

The forms of 2,4-D that are highly toxic to fish include 2,4-D ester formulations, N-oleyl-1,3-propylenediamine salt, and the N,N-dimethyl-oleyl-linoleylamine, compared to LC_{50} concentrations in Table 3-1 (USDA 1995c). The chemical is reported as having a half-life of 2 days in fish and oysters (EXTOXNET 1996a).

The toxicity of 2,4-D to aquatic organisms ranges from practically non-toxic to highly toxic (Table 3-16). The herbicide 2,4-D amine is highly toxic to rainbow trout but practically non-toxic to bluegill, while the butoxyethanol ester is moderately toxic to both rainbow trout and bluegill. Esters are typically 100 times more toxic to aquatic organisms than their corresponding acids and most amine formulations, but in most instances the esters rapidly hydrolyze to corresponding acids (see WSDOT 1993). Bioaccumulation of 2,4-D is low, and it generally is rapidly excreted in the urine unchanged or as a conjugate. 2,4-D amine salt forms are generally non-toxic to fish (USDA 1995c). The relative risks to fish from WSDOT's current use of 2,4-D were calculated to be low in all physiographic provinces of the state examined except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-14).

3.3.2.2 Aquatic Invertebrates

2,4-D compounds most toxic to aquatic invertebrates (e.g. macrophytes, algae) are the ester and dimethyl amine formulations (USDA 1995c). Both 2,4-D amine and 2,4-D butoxyethanol ester are moderately toxic to aquatic invertebrates (WSDOT 1993). 2,4-D is reported as slightly toxic to Dungeness crab, in relation to criteria in Table 3-1 (EXTOXNET 1996a). The relative risks to invertebrates from WSDOT's current use of 2,4-D were calculated to be slight in all physiographic provinces of the state (Table 3-15).

3.4 Ammonium Salt of Fosamine

3.4.1 Exposure Assessment

Exposure for fish and aquatic invertebrates may occur from direct contact with surface water that contains the herbicide due to runoff after application to soils and vegetation. Fosamine does not tend to bioaccumulate in aquatic organisms; therefore, the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced. In a recreation of contamination via drift application, threshold-effect concentrations of Krenite (a formulation of Fosamine) reported in partial life-cycle studies for salmonids are estimated to be less than 75 times the maximum theoretical concentration potentially found in shallow waters due to direct overhead spray application (Swift *et al*. 2002). As demonstrated in Table 3-13, the relative exposure of aquatic animals to Fosamine is estimated to be in the low to moderate range for all physiographic provinces in Washington State, based on application rates and other exposure parameters provided by WSDOT.

3.4.2 Risk Characterization

3.4.2.1 Fish

As previously reported, Fosamine is considered practically non-toxic to fish, based on criteria in Table 3-1 (Tu *et al.* 2001a). The most sensitive stage in salmonids to Fosamine is the yolk-sac fry stage (Swift *et al.* 2002). Based upon limited available evidence, Fosamine has not been reported to bioaccumulate appreciably within aquatic organisms (*see* WSDOT 1993). The relative risks to fish from WSDOT's current use of Fosamine were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.4.2.2 Aquatic Invertebrates

Fosamine is listed as practically non-toxic to aquatic microorganisms and invertebrates (Tu *et al.* 2001a, Swift *et al*. 2002, BPA 2000a). The relative risks to aquatic invertebrates from WSDOT's current use of Fosamine were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.5 Bromacil

3.5.1 Exposure Assessment

Exposure for fish and aquatic invertebrates involves direct contact to surface water that may contain the herbicide due to runoff after application to soils. Bromacil does not bioaccumulate in aquatic organisms, therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources to fish are an incomplete transport function. As demonstrated in Table 3-13, the relative exposure of aquatic animals to Bromacil is estimated to be in the moderate range for all physiographic provinces in Washington State, based on application rates and other exposure parameters provided by WSDOT.

3.5.2 Risk Characterization

3.5.2.1 Fish

Bromacil toxicity to fish varies from practically non-toxic to slightly toxic (WSDOT 1993). Bromacil is metabolized in aquatic organisms to its debrominated analog. It does not bioaccumulate in aquatic organisms. The relative risks to fish from WSDOT's current use of Bromacil were calculated to be low in all physiographic provinces of the state except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-14).

3.5.2.2 Aquatic Invertebrates

Bromacil toxicity to aquatic invertebrates varies from practically non-toxic to slightly toxic (Table 3-10). The relative risks to aquatic invertebrates from WSDOT's current use of Bromacil were calculated to be low in all physiographic provinces of the state except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-15).

3.6 Chlorsulfuron

3.6.1 Exposure Assessment

Exposure for fish and aquatic organisms to Chlorsulfuron can occur through direct contact with surface water that may contain the herbicide due to runoff after application to soils, drift, and other mechanisms previously discussed. Chlorsulfuron does not tend to bioaccumulate in organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources to fish and invertebrates is reduced (WSDOT 1993). As demonstrated in Table 3-13, the relative exposure of aquatic animals to Chlorsulfuron is estimated to be in the low to moderate range for all physiographic provinces in Washington State, based on application rates and other exposure parameters provided by

WSDOT. Exposure is estimated to be the lowest in the Columbia Basin and Blue Mountain regions and greatest in the Puget Trough.

3.6.2 Risk Characterization

3.6.2.1 Fish

Chlorsulfuron is practically non-toxic to fish and does not tend to bioaccumulate. The relative risks to fish from WSDOT's current use of Chlorsulfuron were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.6.2.2 Aquatic Invertebrates

Chlorsulfuron is considered practically non-toxic to aquatic invertebrates (Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Chlorsulfuron were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.7 Clopyralid

3.7.1 Exposure Assessment

Clopyralid is relatively persistent in water, but is also highly soluble (300,000 mg/L at a pH of 7.0 and temperature of 25ºC). It does not bind well to suspended sediments, but will eventually sink because it has a heavier molecular mass than water (192.0), and will degrade in aquatic sediments after reaching the bottom (Tu *et al.* 2001e, IPCS CEC 1993). Clopyralid half-life in water ranges from 8 to 40 days. An experiment conducted by Leitch and Fagg (1985 *as cited in* Tu *et al.* 2001e) estimated the rate of leaching to a nearby stream that occurred after an aerial application of 2.5 kg formulated product/ha. They estimated that 0.01% of Clopyralid leached into the stream after the first significant rainfall event three days after application. Leaching rate depends on:

- Soil characteristics
- Precipitation rate after application
- Distance of stream from application area

Exposure for fish and other aquatic animals primarily occurs through direct contact to surface water that may contain the herbicide due to runoff after application to soils. Clopyralid does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced (BPA 2000b). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Clopyralid would qualify as moderately-low to moderate in all physiographic provinces of Washington State where it may be applied.

3.7.2 Risk Characterization

3.7.2.1 Fish

Clopyralid is reported as practically non-toxic to fish, based on Table 3-1 criteria, with LC_{50} concentrations of 125 mg/L for bluegill sunfish and 104 mg/L for rainbow trout (Tu *et al.* 2001e, Table 3-16). In a separate report, the LC_{50} concentrations after 96 hour exposures were reported as >100 mg/L for both bluegill sunfish and rainbow trout (BPA 2000b). There is little to no potential for bioaccumulation of Clopyralid within aquatic organisms (BPA 2000b). The relative risks to fish from WSDOT's current use of Clopyralid were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.7.2.2 Aquatic Invertebrates

On an acute basis, Clopyralid is considered practically non-toxic to aquatic invertebrates (BPA 2000b, Dow AgroScience 1999a). The relative risks to aquatic invertebrates from WSDOT's current use of Clopyralid were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.8 Clopyralid/2,4-D

3.8.1 Exposure Assessment

Clopyralid/2,4-D is miscible in water (Dow AgroScience 2001). Exposure for fish and other aquatic animals could occur through direct contact in surface water that may contain the herbicide due to runoff after application or drift. Clopyralid/2,4-D does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources to fish is reduced (BPA 2000b). As demonstrated in Table 3-13, the relative exposure of aquatic animals to Clopyralid/2,4-D is estimated to be in the moderate range for all physiographic provinces in Washington State, based on application rates and other exposure parameters provided by WSDOT.

3.8.2 Risk Characterization

3.8.2.1 Fish

The Clopyralid/2,4-D mixture, similar to both Clopyralid and 2,4-D chemicals separately, is considered practically non-toxic to fish (BPA 2000b, BPA 2000d, Table 3-16). There is little to no potential for bioaccumulation of Clopyralid/2,4-D within aquatic organisms (BPA 2000b, BPA 2000d). The relative risks to fish from WSDOT's current use of Clopyralid/2,4-D were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.8.2.2 Aquatic Invertebrates

Clopyralid/2,4-D is moderately toxic to aquatic organisms and the compound Clopyralid is practically non-toxic to aquatic organisms (Dow AgroScience 2001). The relative risks to aquatic invertebrates from WSDOT's current use of Clopyralid/2,4-D active ingredients were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.9 Dicamba

3.9.1 Exposure Assessment

Dicamba is persistent in water and degradation via hydrolysis is minimal (Syngenta 2000). Dicamba breaks down in water mostly via photodegradation, with a half-life of 50 days. The solubility of Dicamba in water is 0.5 percent at 25ºC (6,500 mg/L), and is considered slightly soluble (Syngenta 2000, USDA 1995d). The chemical binds slowly to suspended particles; however, it is degraded quickly in aquatic sediments once it reaches bottom depths.

Exposure for fish and other aquatic animals can occur through direct contact with surface water that may contain the herbicide due to runoff after application to soils. Dicamba does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources to fish has been considered reduced (USDA 1995d). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Dicamba would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.9.2 Risk Characterization

3.9.2.1 Fish

In the studies reviewed, Dicamba is considered slightly to practically non-toxic to fish (Table 3-16). Dicamba appears to be more toxic to sensitive species, such as rainbow trout, than to carp, although there is some variability in the literature of reported LC_{50} values. The salts and free acid of Dicamba are considered toxicologically equivalent because the salt hydrolyzes to the free acid in an aqueous environment (see WSDOT 1993). Based on the available data, Dicamba does not appear to bioaccumulate (USDA 1995d). The relative risks to fish from WSDOT's current use of Dicamba were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.9.2.2 Aquatic Invertebrates

Dicamba is considered practically non-toxic to aquatic invertebrates (Table 3-16). The relative risks to invertebrates from WSDOT's current use of Dicamba were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.10 Dicamba/2,4-D

3.10.1 Exposure Assessment

Similar to Dicamba, Dicamba/2,4-D has two forms: an amine salt and ester. The amine salt formulation (found in commercial products such as Brash) of Dicamba/2,4-D is miscible in water (Terra 1999). Exposure for fish and other aquatic animals involves ingestion of affected aquatic invertebrates or direct contact to surface water that may contain the herbicide due to runoff after application to vegetation and soils. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Dicamba would qualify as moderately-low to moderate in all physiographic provinces of Washington State where it may be applied.

3.10.2 Risk Characterization

3.10.2.1 Fish

Dicamba/ 2,4-D is reported as practically non-toxic to fish, based on Table 3-1 criteria (Tu *et al*. 2001f, Table 3-16). The relative risks to fish from WSDOT's current use of Dicamba/2,4-D were calculated to be low in all physiographic provinces of the state examined (Table 3-14).

3.10.2.2 Aquatic Invertebrates

Dicamba is reported as practically non-toxic to aquatic microorganisms and invertebrates (Tu *et al.* 2001f, Swift *et al.* 2002, BPA 2000e, Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Dicamba/2,4-D were calculated to be low in all physiographic provinces of the state examined (Table 3-15).

3.11 Dicamba/MCPA

3.11.1 Exposure Assessment

Exposure for fish involves ingestion of affected aquatic invertebrate or direct contact to surface water that may contain the herbicide due to runoff and/or drift after application. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Dicamba/MCPA would qualify as moderately low to moderate in all physiographic provinces of Washington State where it may be applied.

3.11.2 Risk Characterization

3.11.2.1 Fish

Dicamba/MCPA is reported as practically non-toxic to fish, based on Table 3-1 criteria (Tu *et al.* 2001f). The relative risks to fish from WSDOT's current use of Dicamba/MCPA were calculated to be low in all physiographic provinces examined (Table 3-14).

3.11.2.2 Aquatic Invertebrates

Dicamba/MCPA is reported as practically non-toxic to aquatic microorganisms and invertebrates (Tu *et al.* 2001f, Swift *et al.* 2002, BPA 2000e). The relative risks to aquatic invertebrates from WSDOT's current use of Dicamba/MCPA were calculated to be low in all physiographic provinces examined (Table 3-15).

3.12 Dichlobenil

3.12.1 Exposure Assessment

Exposure for fish and other aquatic animals is primarily through direct contact to surface water that may contain the herbicide due to runoff and/or drift after application. Dichlobenil does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources to fish is reduced (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Dichlobenil would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.12.2 Risk Characterization

3.12.2.1 Fish

Dichlobenil is considered slightly to moderately toxic to fish and to other aquatic invertebrate animals (USDA 1995e, Table 3-16). Acute toxicity levels of concern were exceeded for endangered fish and aquatic invertebrates at the 20 lb. ai/A rate for unincorporated application (U.S. EPA 1998b). From the limited data available, it does not appear that Dichlobenil bioaccumulates to a great extent in fish. The breakdown product, 2,6-DCBA is practically non-toxic in fish. The relative risks to fish from WSDOT's current use of Dichlobenil were slight in all physiographic provinces examined (Table 3-14).

3.12.3 Aquatic Invertebrates

Dichlobenil is considered moderately to highly toxic to aquatic invertebrates (Table 3-16). As discussed above, exposure of aquatic organisms through direct application of Dichlobenil into a non-flowing water source (*i.e*. pond, lake, reservoir) may pose acute toxicity risks to invertebrates (Table 3-

3). The relative risks to aquatic invertebrates from WSDOT's current use of Dichlobenil reflect this increased toxicity, with ratings of moderate risks in all provinces except 7 and 8, where relative risks where characterized as slight (Table 3-15).

3.13 Diuron

3.13.1 Exposure Assessment

Exposure for fish and other aquatic animals may occur fromingestion of chronically exposed aquatic invertebrates or other food sources, or via direct contact to surface water that may contain the herbicide due to runoff after application. There is some evidence of limited bioaccumulation of Diuron in carp and some aquatic invertebrates upon prolonged exposure (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Diuron would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.13.2 Risk Characterization

3.13.2.1 Fish

The toxicity data indicate that Diuron is considered moderately to highly toxic to fish (Table 3-16). Limited data are available describing the bioaccumulation of Diuron in aquatic organisms. The relative risks to fish from WSDOT's current use of Diuron was slight in the Columbia Basin and Blue Mountain regions and moderate in the other regions of the state (Table 3-14).

3.13.2.2 Aquatic Invertebrates

The toxicity data reviewed indicate that Diuron is moderately to highly toxic to aquatic invertebrates (Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Diuron was low in provinces 7 and 8 and moderate in provinces 1 through 6 (Table 3-15).

3.14 Glyphosate

3.14.1 Exposure Assessment

Exposure for fish is primarily through direct contact to surface water that may contain the herbicide due to runoff after application. Glyphosate does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Glyphosate would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.14.2 Risk Characterization

3.14.2.1 Fish

Glyphosate is considered practically non-toxic to moderately toxic to fish (Table 3-16). The available toxicity data do not indicate that it bioaccumulates. The relative risks to fish from WSDOT's current use of Glyphosate was low in all physiographic provinces examined except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-14).

3.14.2.2 Aquatic Invertebrates

Glyphosate is considered slightly to practically non-toxic to aquatic invertebrates (Table 3-16). The available toxicity data do not indicate that it bioaccumulates. The relative risks to aquatic invertebrates from WSDOT's current use of Glyphosate was low in all physiographic provinces examined except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-15).

3.15 Metsulfuron Methyl

3.15.1 Exposure Assessment

Exposure for fish is primarily through direct contact to surface water that may contain the herbicide due to runoff after application to soils. Bioaccumulation of Metsulfuron methyl in aquatic organisms is unlikely based on metabolism in water and octanol/water partition coefficient; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Metsulfuron Methyl would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.15.2 Risk Characterization

3.15.2.1 Fish

Metsulfuron methyl is practically non-toxic fish. No information was found describing its metabolism or bioaccumulation in aquatic species, but we can predict (based on metabolism and the octanol/water partition coefficient) that bioaccumulation is unlikely to occur. The relative risks to fish from WSDOT's current use of Metsulfuron was low in all physiographic provinces examined (Table 3-14).

3.15.2.2 Aquatic Invertebrates

Metsulfuron methyl is practically non-toxic aquatic invertebrates (Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Metsulfuron were low in all physiographic provinces examined (Table 3-15).

3.16 Oryzalin

3.16.1 Exposure Assessment

Exposure for fish and other aquatic animals is primarily through direct contact to surface water that may contain the herbicide due to runoff after application. Bioaccumulation of Oryzalin in aquatic organisms is unlikely based on metabolism in water and a relatively low octanol/water partition coefficient; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Oryzalin would qualify as moderately low to moderate to in all physiographic provinces of Washington State where it may be applied.

3.16.2 Risk Characterization

3.16.2.1 Fish

Oryzalin is moderately toxic to fish. No information was found describing its metabolism or bioaccumulation in aquatic species, but we can predict (based on metabolism and the octanol/water partition coefficient) that bioaccumulation is unlikely to occur (WSDOT 1993). The relative risks to fish from WSDOT's current use of Oryzalin was slight in all physiographic provinces examined (Table 3-14).

3.16.2.2 Aquatic Invertebrates

Oryzalin is considered slightly to highly toxic to aquatic invertebrates (Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Oryzalin were low in all physiographic provinces except for the Puget Trough, where the relative risk was calculated to be slight (Table 3-15).

3.17 Picloram

3.17.1 Exposure Assessment

Exposure for fish and other aquatic animals is primarily through direct contact to surface water that may contain the herbicide due to runoff and/or drift after application. Bioaccumulation of Picloram in aquatic organisms is reported as not occurring; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources is an incomplete transport pathway (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Picloram would qualify as moderate to moderately high in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure was relatively high (2.5).

3.17.2 Risk Characterization

3.17.2.1 Fish

Picloram is slightly to moderately toxic to fish, according to Table 3-1 criteria (Tu *et al.* 2001d, WSDOT 1993). However, reported LC_{50} concentrations are above peak runoff concentrations reported in various studies under environmental conditions (Tu *et al.* 2001d). Mayes et al. (1987 *as cited in* Tu *et al.* 2001d) stated that Picloram is not toxic to rainbow trout life stages by acute or chronic standards when used as directed. Picloram does not bioaccumulate. When using the median toxicity rating from past studies, as conducted in the original EIS, the relative risks to fish from WSDOT's current use of Picloram was low in the Columbia Basin and Blue Mountains provinces, where precipitation and road density are amongst the lowest in the state; in all other regions the relative risk would be considered slight (Table 3-14).

3.17.2.2 Aquatic Invertebrates

Picloram is practically non-toxic to moderately toxic to aquatic invertebrates (WSDOT 1993). Chronic toxicity tests on *Daphnia magna* reported an LC_{50} of 68.3 mg/L, which is within the slightly toxic range depicted in Table 3-1. When using the median toxicity rating from past studies, as conducted in the original EIS, the relative risks to aquatic invertebrates from WSDOT's current use of Picloram was slight in physiographic provinces 1 through 6 and low in the Columbia Basin and Blue Mountains provinces (Table 3-15), where precipitation and road density are amongst the lowest in the state.

3.18 Sulfometuron Methyl

3.18.1 Exposure Assessment

Exposure for fish is primarily through direct contact to surface water that may contain the herbicide due to runoff and/or drift after application. Bioaccumulation of Sulfometuron methyl in aquatic organisms is reported as not occurring; therefore the potential of exposure through ingestion of exposed aquatic invertebrates or other food sources was considered low (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Sulfometuron methyl would qualify as low in most physiographic provinces of Washington State where it may be applied, except in the Puget Trough, where exposure would be considered moderate, and in the Columbia Basin and Blue Mountains, where exposure would be considered low.

3.18.2 Risk Characterization

3.18.2.1 Fish

Sulfometuron methyl is slightly toxic to fish (Table 3-16). Studies indicate that Sulfometuron methyl does not bioaccumulate in aquatic species. The relative risks to fish from WSDOT's current use of Triclopyr was low in all physiographic provinces examined (Table 3-14).

3.18.2.2 Aquatic Invertebrates

Sulfometuron methyl is practically non-toxic to slightly toxic to aquatic invertebrates (Table 3-16). The relative risks to aquatic invertebrates from WSDOT's current use of Sulfometuron methyl was low in all physiographic provinces examined (Table 3-15).

3.19 Triclopyr

3.19.1 Exposure Assessment

There are two main forms of Triclopyr found in commercial formulations: amine salt and ester. Of the two forms, the ester is the more toxic to aquatic environments. Both forms rapidly break down to a less toxic form under normal conditions in water via sunlight (USDA 1995b). The half-life of Triclopyr in water is less than 24 hours (USDA 1995b). Triclopyr solubility in water is reported as moderate to low (USDA 1995b). Exposure for fish and other aquatic animals involves ingestion of affected aquatic invertebrate or other food sources or direct contact to surface water that may contain the herbicide due to runoff/drift after application. Triclopyr does not tend to bioaccumulate in aquatic organisms; therefore the potential of exposure through ingestion of exposed aquatic organisms or other food sources is reduced (WSDOT 1993). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Triclopyr would qualify as moderate in all physiographic provinces of Washington State where it may be applied, except in provinces 7 and 8, where exposure would be considered moderately low.

3.19.2 Risk Characterization

3.19.2.1 Fish

As summarized in Table 3-16, the toxicity of Triclopyr to fish ranges from practically non-toxic to highly toxic (USDA 1989). Rapid excretion of Triclopyr suggests that there is low potential for bioaccumulation. The parent compound and amine salt is practically non-toxic to fish. According to Table 3-1 criteria, however, the ester formulation in Garlon 4, has a lower LC_{50} concentration, and is considered highly toxic to fish (0.1 to 1 mg/L) (EXTOXNET 1996e, USDA 1995b). The relative risks to fish from WSDOT's current use of Triclopyr was slight in all physiographic provinces examined (Table 3-14).

3.19.2.2 Aquatic Invertebrates

Triclopyr amine salt is practically non-toxic to aquatic invertebrates, according to Table 3-1 criteria and summarized in Table 3-10 (USDA 1989, EXTOXNET 1996e). No values for response to the ester formulation of Triclopyr were provided. The relative risks to aquatic invertebrates from WSDOT's current use of Triclopyr were low in all physiographic provinces examined (Table 3-15).

Herbicides Evaluated in 2005

3.20 Bromoxynil

3.20.1 Exposure Assessment

Exposure for fish and other aquatic animals primarily involves direct contact with surface water that may contain the herbicide due to runoff/drift following applications. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Bromoxynil is mobile in sand, sandy load and loam soils but is not expected to persist in surface or ground water. Bromoxynil is unlikely to bioaccumulate in aquatic organisms; therefore, the potential for exposure through ingestion of exposed aquatic invertebrates or other food sources is limited (U.S. EPA 1998a). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Bromoxynil would qualify as moderately low in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderate, and the Columbia Plateau and Blue Mountain provinces, where exposure would be considered low.

3.20.2 Risk Characterization

3.20.2.1 Fish

Bromoxynil octanoate is considered highly to very highly toxic to fish based on results from acute toxicity tests as summarized in Table 3-16. Bromoxynil is not expected to persist in surface waters or to bioaccumulate in fish. The relative risks to fish from application of Bromoxynil at levels established by WSDOT were slight in the Columbia Plateau and Blue Mountain provinces and moderate in all other physiographic provinces of the state (Table 3-14). Risks to fish from Bromoxynil application are moderated by its low persistence and relatively low application rate.

3.20.2.2 Aquatic Invertebrates

Bromoxynil toxicity to aquatic invertebrates varies from moderately toxic to very highly toxic (Table 3-16). The relative risks to aquatic invertebrates from application of Bromoxynil at levels established by WSDOT were slight in the Columbia Plateau and Blue Mountain provinces and moderate in all other physiographic provinces of the state (Table 3-15). Like fish, risks to aquatic invertebrates from Bromoxynil application are moderated by its low persistence and relatively low proposed application rate.

3.21 Diflufenzopyr

3.21.1 Exposure Assessment

Exposure for fish involves direct contact with surface water that may contain the herbicide due to runoff following herbicide application. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Diflufenzopyr is not persistent in the environment (see Chapter 2 Section 3) thus limiting the potential exposure to fish. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Diflufenzopyr would qualify as moderately low in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderate, and the Columbia Plateau and Blue Mountain provinces (*i.e.*, 6, 7), where exposure would be considered low.

3.21.2 Risk Characterization

3.21.2.1 Fish

Acute toxicity of Diflufenzopyr to fish ranged from low to practically non-toxic as summarized in Table 3-10. Due to its low toxicity, limited persistence and relatively low application rate, the estimated risk to fish from the application of Diflufenzopyr at levels established by WSDOT is low in all physiographic provinces of the state examined (Table 3-14).

3.21.2.2 Aquatic Invertebrates

Acute toxicity of Diflufenzopyr to aquatic invertebrates ranged from low to practically non-toxic as summarized in Table 3-16. Due to its low toxicity, limited persistence and relatively low application rate, the estimated risk to aquatic invertebrates from the application of Diflufenzopyr at levels established by WSDOT is low in all physiographic provinces of the state examined (Table 3-15).

3.22 Flumioxazin

3.22.1 Exposure Assessment

Exposure for fish and other aquatic animals can result from spray drift from aerial applications or from runoff following aerial or surface applications. Flumioxazin is not highly persistent in the environment, thus limiting the potential exposure to fish (see Chapter 2, Section 3). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Flumioxazin would qualify as moderately low in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderate, and the Columbia Plateau and Blue Mountain provinces, where exposure would be considered low.

3.22.2 Risk Characterization

3.22.2.1 Fish

Flumioxizan is considered slightly to moderately toxic to fish as summarized in Table 3-16. Due primarily to its low persistence and relatively low application rate, the estimated risks to fish from Flumioxazin applied at levels established by WSDOT are slight in all physiographic provinces of the state examined, except in the Columbia Plateau and Blue Mountain regions, where the risks would be characterized as low (Table 3-14).

3.22.2.2 Aquatic Invertebrates

Flumioxizan is considered moderately toxic to freshwater invertebrates and moderately to highly toxic to marine/estuarine invertebrates as summarized in Table 3-16. Due primarily to its low persistence and relatively low application rate, the estimated risks to aquatic organisms from Flumioxazin applied at levels established by WSDOT are slight in all physiographic provinces of the state examined (Table 3-15).

3.23 Fluroxypyr

3.23.1 Exposure Assessment

Exposure for fish and other aquatic animals can result from spray drift from aerial applications or from runoff following aerial or surface applications. Fluroxopyr is highly mobile in soil but its persistence is limited due to dissipation from hydrolysis and microbial degradation (see Chapter 2, Section 3); these factors limit potential exposure to fish and aquatic invertebrates. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Fluroxypyr would qualify as moderate in all physiographic provinces of Washington State where it may be applied.

3.23.2 Risk Characterization

3.23.2.1 Fish

Fluroxypyr is slightly toxic to practically non-toxic to fish as summarized in Table 3-16. Due to its relatively low toxicity, persistence and application rate, the estimated risk to fish from Fluroxypyr applied at levels established by WSDOT was calculated to be low in all physiographic provinces of the state examined, except in the Puget Trough, where the risk was characterized as slight (Table 3-14).

3.23.2.2 Aquatic Invertebrates

Fluroxypyr toxicity to aquatic invertebrates was found to range from practically non-toxic to very highly toxic as summarized in Table 3-16. Based on this range, a moderate toxicity was assumed for the evaluation of relative risks. The relative risks to aquatic invertebrates from Fluroxypyr applied at levels established by WSDOT were calculated to be slight in all physiographic provinces of the state examined (Table 3-15) except for the Columbia Plateau and Blue Mountain regions where risks are low. Despite assuming moderate toxicity, the low persistence and low application rate of Fluroxypyr limited the estimated risk to aquatic invertebrates.

3.24 Imazapyr

3.24.1 Exposure Assessment

Exposure for fish and other aquatic animals can result from spray drift from aerial applications or from runoff following aerial or surface applications. Imazapyr is highly persistent in soil but breaks down relatively quickly in water, thus limiting the potential for exposure among fish (See Chapter 2 Section 3). Since the exposure for this evaluation was based on the persistence characteristics of Imazapyr in soil, the estimated exposure to fish is highly conservative. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Imazapyr would qualify as moderate in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderately high.

3.24.2 Risk Characterization

3.24.2.1 Fish

Despite its high persistence and moderately high proposed application rate, the estimated risk to fish from Imazapyr applied at levels established by WSDOT is low in the Columbia Plateau and Blue Mountain regions and slight in the other six regions of the state (Table 3-14). Risks from Imazapyr application are limited because it is only slightly toxic to fish, as summarized in Table 3-16.

3.24.2.2 Aquatic Invertebrates

Imazapyr is slightly toxic to aquatic invertebrates as summarized in Table 3-16. Despite its high persistence and moderately high proposed application rate, the estimated risk to aquatic invertebrates from Imazapyr applied at levels established by WSDOT is low in the Columbia Plateau and Blue Mountain regions and slight in the other six regions of the state (Table 3-15).

3.25 Isoxaben

3.25.1 Exposure Assessment

Exposure for fish and other aquatic animals can result from spray drift from aerial applications or from runoff following aerial or surface applications. Isoxaben is highly persistent in soil but breaks down relatively quickly in water due to photolysis (See Chapter 2 Section 3). Limited persistence of Isoxaben in water would likely decrease the exposure of aquatic invertebrates. Since this assessment based the persistence factor on the more conservative soil data, it is likely that exposure to fish and other aquatic animals is overestimated. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Isoxaben would qualify as moderate in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderately high.

3.25.2 Risk Characterization

3.25.2.1 Fish and Aquatic Invertebrates

No toxicity data for fish or aquatic invertebrates was identified; thus risks from application of Isoxaben were not calculated.

3.26 Norflurazon

3.26.1 Exposure Assessment

Exposure for fish and aquatic animals could result from spray drift from aerial applications or from runoff following aerial or surface applications. Norflurazon is unlikely to bioaccumulate in aquatic organisms; therefore, the potential for exposure through ingestion of exposed aquatic invertebrates or other food sources is limited. Norflurazon is persistent both in aerobic and anaerobic aquatic environments and is resistant to hydrolysis, which increases the exposure potential for fish (See Chapter 2 Section 3). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Norflurazon would qualify as moderate in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderately high.

3.26.2 Risk Characterization

3.26.2.1 Fish

Norflurazon is considered slightly to moderately toxic to fish based on results from acute toxicity tests evaluated by U.S. EPA (1996), as summarized in Table 3-16. The relative risks to fish from Norflurazon applied at levels established by WSDOT were calculated to be slight in all physiographic provinces except in the Puget Trough where the risk is moderate (Table 3-14).

3.26.2.2 Aquatic Invertebrates

Norflurazon toxicity to aquatic invertebrates varies from slightly toxic to moderately toxic (Table 3-16) The relative risks to aquatic invertebrates from Norflurazon applied at levels established by WSDOT were calculated to be slight in all physiographic provinces except in the Puget Trough where the risk is moderate (Table 3-15).

3.27 Oxadiazon

3.27.1 Exposure Assessment

Exposure for fish and aquatic invertebrates is primarily through direct contact to surface water that may contain the herbicide due to runoff/drift after application. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Due to its strong affinity for soil and limited mobility, Oxadiazon has the potential to be transferred to surface water following rainfall events, thus increasing the potential for exposure among fish and other aquatic animals (U.S. EPA 2003). Potential exposure of aquatic invertebrates to Oxadiazon is enhanced due to its relatively high mobility and persistence in soil and water (U.S. EPA 2003). As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Oxadiazon would qualify as moderate in all physiographic provinces of Washington State where it may be applied, except the Puget Trough, where exposure would be considered moderately high.

3.27.2 Risk Characterization

3.27.2.1 Fish

Oxadiazon is considered moderately to highly toxic to fish. U.S. EPA conducted an independent Tier 1 risk assessment (GENEEC) that suggests an elevated risk for freshwater and marine estuarine fish (Risk Quotient > 0.1) (U.S. EPA 2003). The relative risks to fish from application of Oxadiazon at levels established by WSDOT were considered moderate for all physiographic provinces (Table 3-14)

3.27.2.2 Aquatic Invertebrates

Oxadiazon is considered moderately to highly toxic to aquatic invertebrates. The relative risks to aquatic invertebrates from application of Oxadiazon at levels established by WSDOT were considered moderate across all physiographic provinces (Table 3-15).

3.28 Pendimethalin

3.28.1 Exposure Assessment

Exposure for fish and other aquatic animals is primarily through direct contact to surface water that may contain the herbicide due to runoff after application to soils, or from runoff following aerial or surface applications. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Environmental persistence of Pendimethalin varies widely depending on characteristics of the media in which it is found. Due to its strong affinity for soil and limited mobility, Pendimethalin has the potential to be transferred to surface water following rainfall events. Pendimethalin has a high potential to bioaccumulate in fish tissues with bioaccumulation factors of 1400 times for edible tissue and 5100 times for whole fish, thus increasing the potential exposure to fish via ingestion of aquatic organisms (U.S. EPA 1997) (See Chapter 2 Section 3). Due to its persistence in soil and its potential for bioaccumulation in aquatic organisms, the potential exposure of Pendimethalin to aquatic invertebrates is also enhanced, particularly in areas with high rainfall and road density. As demonstrated in Table 3-13, the estimated relative exposure of aquatic animals to Pendimethalin would qualify as moderate in all physiographic provinces of Washington State except the Puget Trough, where exposure would be considered moderately high.

3.28.2 Risk Characterization

3.28.2.1 Fish

Pendimethalin is considered highly toxic to fish as summarized in Table 3-16. The relative risks to fish from application of Pendimethalin at levels established by WSDOT were moderate in all physiographic provinces examined (Table 3-14).

3.28.2.2 Aquatic Invertebrates

Pendimethalin is considered highly toxic to aquatic invertebrates as summarized in Table 3-16. The relative risks to aquatic invertebrates from application of Pendimethalin at levels established by WSDOT were moderate in all physiographic provinces.

3.29 Pyraflufen

3.29.1 Exposure Assessment

Exposure for fish and aquatic invertebrates can result from spray drift from aerial applications or from runoff following aerial or surface applications. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Information on environmental fate of Pyraflufen was not available in the sources searched for this evaluation (see Section 1.2); thus, a conservative assumption was made that Pyraflufen is highly persistent. Exposure was considered moderate in all physiographic provinces.

3.29.2 Risk Characterization

3.29.2.1 Fish

Pyraflufen toxicity to fish ranges from practically non-toxic to highly toxic as summarized in Table 3-16. Based on this range of toxicity, a moderate toxicity level was selected for this assessment. In addition, a conservative assumption was made with regard to the persistence of Pyraflufen. Nevertheless, primarily because of its low application rate (see Chapter 2 Table 3-2), the estimated risk to fish from using Pyraflufen at levels established by WSDOT were slight in all physiographic provinces of the state examined, except in the Columbia Plateau and Blue Mountain regions, where the risks would be characterized as low (Table 3-14).

3.29.2.2 Aquatic Invertebrates

Pyraflufen toxicity to aquatic invertebrates ranges from practically non-toxic to highly toxic as summarized in Table 3-16. Based on this range of toxicity, a moderate toxicity level was selected for this assessment. In addition a conservative assumption was made with regard to the persistence of Pyraflufen. Nevertheless, primarily because of its low application rate (Chapter 2 Table 3-2), the estimated risk to aquatic invertebrates from using Pyraflufen at levels established by WSDOT were slight in all physiographic provinces of the state examined, except in the Columbia Plateau and Blue Mountain regions, where the risks would be characterized as low (Table 3-15).

3.30 Sulfentrazone

3.30.1 Exposure Assessment

Exposure for fish and aquatic invertebrates can result from spray drift from aerial applications or from runoff following aerial or surface applications. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Sulfentrazone is persistent and highly mobile and has a strong potential to migrate off site (see Chapter 2 Section 3). These factors increase the likelihood of potential exposure to fish. Environmental persistence and a high degree of mobility also increase the potential for exposure to Sulfentrazone among aquatic invertebrates. The relative exposure for sulfentrazone was considered moderate for all physiographic provinces (Table 3-13).

3.30.2 Risk Characterization

3.30.2.1 Fish

Despite having a relatively high persistence in soil and water, the estimated risks to fish from application of Sulfentrazone at levels established by WSDOT were low in all physiographic provinces, except for the Puget Trough, where the risk was considered slight (Table 3-14). The limited risk from Sulfentrazone is due to its relatively low toxicity (Table 3-16) and low application rate (see Chapter 2 Table 3-2).

3.30.2.2 Aquatic Invertebrates

Despite having a relatively high persistence in soil and water, the estimated risks to aquatic invertebrates from application of Sulfentrazone at levels established by WSDOT were low in all physiographic provinces except for the Puget Trough, where the risk was considered slight (Table 3-15). The limited risk from Sulfentrazone is due to its relatively low toxicity (Table 3-16) and low application rate (see Chapter 2 Table 3-2).

3.31 Tebuthiuron

3.31.1 Exposure Assessment

Exposure for fish is primarily through direct contact to surface water that may contain the herbicide due to runoff after application. Exposure for aquatic invertebrates involves ingestion of exposed phytoplankton or direct contact to surface water that may contain the herbicide due to runoff after application to soils. Exposure could also occur through contact with herbicide that has migrated into aquatic sediment. Tebuthiuron is persistent both in soil and aquatic environments, which increases the potential for exposure among fish and aquatic invertebrates. Tebuthiuron does not tend to bioaccumulate in aquatic organisms; therefore the potential for exposure through ingestion of exposed aquatic invertebrates or other food sources is reduced (USDA 1995g). The relative exposure for sulfentrazone appears moderate for all physiographic provinces, except the Puget Trough, where it could be considered moderately high (Table 3-13).

3.31.2 Risk Characterization

3.31.2.1 Fish

Tebuthiuron is considered practically non-toxic to slightly toxic to fish as summarized in Table 3-10. The relative risks to fish based on application of Tebuthiuron at levels established by WSDOT were slight in all physiographic provinces except for provinces 6 and 7, where the risk was considered low, primarily due to reduced road densities and rainfall in these provinces (Table 3-14). The limited risk posed to fish by Tebuthiuron is primarily due to its low toxicity (Table 3-16).

U.S. EPA, in a separate assessment concluded that use of Tebuthiuron would not pose an unacceptable risk to aquatic organisms if it was applied to a given location no more than one time in a three year cycle (U.S. EPA 1994).

3.31.2.2 Aquatic Invertebrates

Tebuthiuron is considered practically non-toxic to slightly toxic to aquatic invertebrates. The relative risks to aquatic invertebrates based on application of Tebuthiuron at levels established by WSDOT were slight in all physiographic provinces except for provinces 6 and 7, where the risk was considered low, primarily due to reduced road densities and rainfall in these provinces (Table 3-15). The limited risk posed to aquatic invertebrates by Tebuthiuron is primarily due to its low toxicity (Table 3-16).

4.0 Uncertainties and Data Gaps

4.1 Adjuvant & Inert Ingredients

As demonstrated in Section 2.30, little remains known about the potential effects of adjuvants and inert ingredients on the aquatic toxicity of herbicides applied to roadside areas by the WSDOT. Many of the products used do not list the adjuvants and carriers that are integral to the formulations, so it was not possible to ascertain their respective toxicities. However, toxicity tests referenced were conducted with the complete product, so the effects of the inert ingredients within the product formulations should be inherently addressed. The toxicity of added surfactants and other adjuvants that facilitate uptake or efficacy, however, has been rarely considered.

A recent unpublished study, conducted by University of Washington Researchers, highlights the need to examine the potential effects of adjuvants and inert ingredients with greater emphasis (Smith *et al.* 2003, submitted). In this rainbow trout study, dose-response curves were developed for four surfactants, R-11 (Wilbur-Ellis Co., Fresno, CA), LI 700 (Loveland Industries, Inc., Greeley, CO), Agri-Dex (Helena Chemical Co., Memphis, TN), and Hasten (Wilbur-Ellis, Co.). Two of the surfactants are currently in use within the state (R-11 and LI 700). Although the U.S. EPA classification for the two surfactants in use is "minimal concern" (USDA 1997), these two surfactant formulations proved the most toxic of the four tested, with LC_{50} concentrations of 6 and 17 mg/L for the R-11 and LI 700 surfactants, respectively. In contrast, Hasten and Agridex, which have yet to receive U.S. EPA classifications, yielded LC_{50} concentrations of 17 and 271 mg/L, respectively. The toxicity of the two surfactants currently in use exceeds that of several of the herbicides currently applied by WSDOT.

4.2 Exposure Uncertainty

There is also substantial uncertainty associated with the parameters used to estimate exposure. As conducted previously (WSDOT 1993), surface runoff was the only exposure mechanism considered, but spray drift could account for a limited amount of additional exposure. Surface runoff may be overestimated in low rainfall areas. Additionally, other sources of herbicide input were not considered when estimating exposure such as run-off caused by residential use. Residential use of herbicides and pesticides has been shown to contribute substantially to the burden of these chemicals in aquatic environments (Schultz *et al*. 2000). There is therefore potential for additive and/or synergistic toxicity that could not be addressed in this analysis. The Department of Ecology does not routinely monitor herbicide concentrations; however, a monitoring program led by the USGS identified detectable levels of herbicides in urban run-off in Washington State, including some of those used by WSDOT (see USGS 1999, www.dwatcm.wr.usgs.gov/ps.nawqa.html). In the USGS study, 2,4-D, Bromacil, Dichlobenil, MCPA and Trichlopyr were occasionally detected in some of the surface water samples collected. Diuron, Dicamba and Picloram were not detected in the monitoring. Several of the herbicides used by WSDOT were not monitored by the USGS, including: Fosamine, Oryzalin, Chlorsulfuron, Clopyralid, Sulfo Meturonmethyl, and Glyphosate, so evidence of potential run-off of these herbicides is still lacking. It is likely that Glyphosate would be detectable given its very high residential use.

The source of the herbicides detected by the USGS was concluded to be primarily from residential sales and use. However, additional run-off contributions, such as from WSDOT's roadside vegetation management program, cannot be precluded, and could conservatively be assumed incorporated in the results. In the USGS study, sampling was conducted in April and May during both peak residential application periods, and peak run-off. Thus, the study was designed to capture the worst-case scenario. Of the WSDOT herbicides used, only 2,4-D was regularly detected (about 2/3rds of the samples, with a range of 0.027 to 1 µg/L). None of the herbicides used by WSDOT have acute or chronic standards, so the detectable levels of the herbicides in the USGS study were not compared against a promulgated standard, and the agency appropriately concluded that without evidence of exposure duration, estimation of risk was not possible. However, a comparison of the monitoring data to the acute toxicity thresholds reported in this assessment suggest that fish and aquatic invertebrate exposure to the run-off in these "worst-case" scenarios would be two to three orders of magnitude below acute toxicity thresholds in the Puget Trough, where the potential for exposure to herbicides applied by WSDOT is considered the highest. For example, the peak concentration of 2,4-D detected by the USGS, 1 ug/L, is three orders of magnitude lower than the reported LC_{50} in cutthroat trout for this herbicide. Some insecticides monitored by the USGS did exceed aquatic life use standards, but these compounds are not used by WSDOT in their integrated pest management program.

Controlled experiments conducted in western Oregon revealed that concentrations of Glyphosate and Diuron were measurable at nearly 1 mg/L after a heavy rainfall, and "a few hundred" µg/L of

Sulfometuron-methyl were also measurable (Wood 2001). In the Oregon study, Diuron was also detectable during the lowest flow month at 0.1 to 0.3 ug/L in a nearby stream that received the drainage ditch conveyance. These results from a neighboring state with similar climate and similar herbicide use practices, highlight how these two herbicides, not detected or measured (Glyphosate) in the USGS Washington study, may also enter public waterways and must be considered for their risks to water-dependent ecological receptors. Collectively, the USGS results in both Oregon and Washington help to emphasize how the estimates of relative risks provided in this assessment would be improved by a site-specific study to confirm or refute exposure parameters.

Another major source of uncertainty relative to the exposure assessment is the exposure duration. For example, mobile organisms may avoid or leave a treated area, and herbicide applications may vary from once a year to once every 2-4 years per site. The methods applied here considered only acute exposure conditions that could result in lethality. Although acute exposures would be most reflective of the use of herbicides by WSDOT, lethal endpoints are not the only means by which aquatic animals could be affected. Sublethal endpoints such as reduced growth and reproductive failure are more common with chronic exposure conditions, but have been documented with some acute exposures to non-herbicide toxicants.

4.3 Toxicity to Atypical Test Species and Effects of Mixtures

Toxicity information available for this supplemental report did not reflect data for many of the aquatic species of greatest interest to Washington State—particularly those managed under the Endangered Species Act (see below for additional disscusson). Similarly, this assessment approach could not consider potential effects on Essential Fish Habitat, as considered under the Magnusen Fisheries Conservation Act. Toxicity information used to assess risk focuses on individual species, and not populations or communities. The overall effects on populations and communities could be significant from the use of herbicides by affecting vegetation components of aquatic habitat, irrespective of toxicity to aquatic animals. Finally, this assessment was primarily focused on individual chemicals, but ecological receptors are often exposed to mixtures in the environment. The state of knowledge on the toxicity of mixtures suggests that additivity is more likely than synergism, but regardless, the elements of additivity and synergism have been evaluated to only a minimal extent.

One recent unpublished study provides some information on the potential for herbicide mixture additivity and/or synergism (Pan *et al*. 2002). In this study, rainbow trout and periphyton were separately exposed to logarithmically increasing doses of 0.1 to 100 ug/L of Roundup (Glyphosate), Krovar (a Bromacil and Diuron mixture), and Oust (Sulfo-meturon methyl). Exposures were conducted using U.S. EPA standard protocols both with the individual chemicals and with a nominally balanced mixture of the three herbicide products. For the mixture, however, the exposure concentrations were 0, 2.6, 26 and 260 mg/L. The rainbow trout used in the assays were at the sensitive swim-up fry stage, but no significant differences in survival were measured at any concentration tested for any herbicide or mixture. A slight and significant reduction in growth was measured at the highest and second to lowest mixture concentration, but the irregular dose-response for this endpoint calls into question the validity of the result. Not surprisingly, the mixture of the herbicides resulted in significant decreases in chlorophyll a, cell density, and relative abundance of Cyanophyta, Chlorophyta, and Cocconeis spp. diatoms in the chronic periphyton assays. Each of the individual herbicides yielded similar significance levels of effects as the mixture relative to the control group with the exception of the chlorophyll assay. On closer inspection, however, the data revealed that the highest concentration of Krovar (100 ug/L) actually yielded a greater depression of chlorophyll a than the highest concentration of mixture tested (260 ug/L). Thus, even though the actual test concentration of the highest concentration of the mixture was 2.6-fold higher than the highest concentration of any of the individual herbicides tested, there was no conclusive evidence of either an additive or synergistic effect using the periphyton assays with this group of herbicides.

4.4 Herbicide Hazards to Threatened and Endangered Aquatic Species of Washington

Resident fish populations managed by the USFWS under the Endangered Species Act (ESA) are delineated as "distinct population segments" (DPS), while the NOAA-Fisheries, which manages marine and anadromous ESA-listed stocks, delineates populations as "evolutionarily significant units" (ESUs). These DPS and ESUs may have exposure to the herbicides applied by WSDOT, as part of their integrated pest management program, thus, it is important to consider how such populations may be affected relative to other aquatic animal populations that have been more routinely studied. However, due to the status of the DPS and ESU stocks, few, if any, direct tests have been conducted to evaluate toxicity of pesticides and herbicides, and information on the toxicity of the compounds to these species must be addressed using surrogate species. Addressing the uncertainty posed by using surrogate test species that may or may not be as sensitive as the threatened and endangered (T&E) populations unique to an area is always problematic in ecological risk assessments. For example, Mayer and Ellersieck (1986), in their compilation of an acute toxicity database for 410 chemicals tested on aquatic organisms, found that toxicity amongst species could range by as much as five orders of magnitude, and for a given species, toxicity could range by as much as 9 orders of magnitude.

Studies conducted in the early 1970s examined the sensitivity of four fish families to 65 different chemicals (Macek and McAllister 1970); salmonids were the most sensitive of the four families (12 species) represented. A more recent study by Sappington *et al*. (2000) evaluated the comparative sensitivity of eight ESA-listed fish species to standard test organisms exposed to five different pesticides or metals in order to validate the use of surrogate species as a predictive tool in toxicilogical assessments. Acute 96-hr exposure trials were conducted, but none of the chemicals tested by these authors were herbicides, and all but nonylphenol had had significant previous testing. The sensitivity of listed cold-water species tested (Apache trout, Lahontan cutthroat trout, greenback cutthroat trout) did not differ significantly after 96-hr exposures from rainbow trout for copper, nonylphenol, or carbaryl. However, they were significantly more sensitive to the organophosphate Permethrin and pentachlorophenol than the rainbow trout. Toxicities exhibited throughout the testing varied with chemical, with some listed species exhibiting greater or lesser sensitivity than the standard test species at some time points (*e.g.*, 12 hours). Although differences were documented which were sometimes statistically significant depending on the time point, the listed species were not always the most sensitive. Most importantly, the maximum degree of difference recorded was less than two-fold, except pentachlorphenol and Permethrin for which the listed species exhibited LC_{50} concentrations less than half of the surrogate rainbow trout. The authors concluded that a safety factor of two would provide a conservative estimate in risk assessments for listed cold-water, warm-water and euryhaline fish species based on these findings.

Another common criticism of ecological risk assessments relying on surrogate species to address potential T&E species effects is the lack of data on sublethal endpoints of site-specific relevance. For example, coastal roadways where herbicides could be applied by WSDOT to control weeds (*e.g.*, like *Spartina*) often drain into dendritic channels that serve as primary staging areas for salmon smolts that are migrating to the sea to mature. The brackish salinties found in the estuaries provide a range of salinities salmon smolts use to adapt to full strength sea-water. The osmoregulatory capacity has been used as one test to establish whether a chemical might affect this sensitive life stage. Patten (2003) examined this capacity, measured as plasma sodium level and gill ATPase activity in a 24-hr seawater challenge, in chinook salmon smolts exposed to Imazapyr concentrations up to 1.6 mg/L (Figure 3-1). This maximum test concentration was over 470-fold greater than the maximum water concentration recovered in the companion study where Imazapyr was applied to bare-mud and measured in waters from the first tidal wash (Patten 2003). As demonstrated in Figure 3-1, there was no consistent dose-response effect recorded on these endpoints of sublethal physiological relevance.

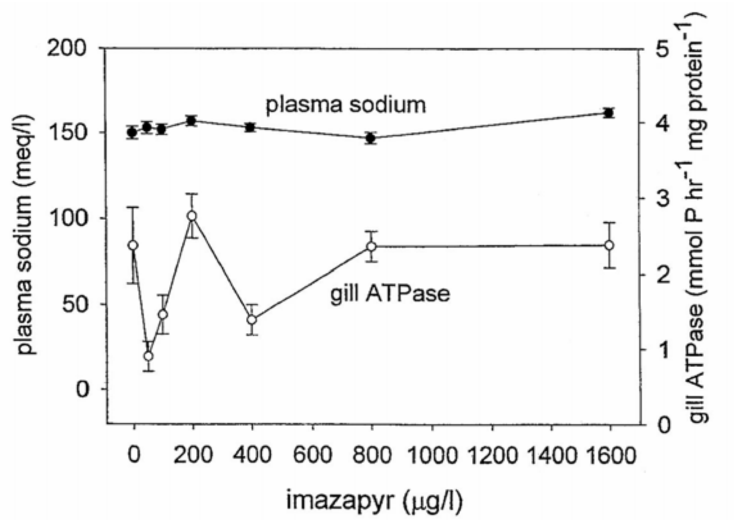

Figure 3-1: Plasma sodium and gill ATPase activity of chinook salmon exposed to Imazapyr (source: Patten 2003).

5.0 Mitigation Measures for Herbicide Application to Avoid Aquatic Risks

The following measures should be considered to minimize aquatic risk from the application of herbicide along roadsides by the WSDOT.

- Do not apply directly to water or areas where surface water is present, or to intertidal areas below the mean high water mark (BPA 2000a).
- Do not apply to irrigation banks or other ditch banks (BPA 2000a).
- Do not apply during periods of rainfall, wind, or expected heavy rainfall.

Table 3-11. Relative Annual Precipitation and Relative WSDOT Road Densities by Physiographic Province.

Physiographic Province	Annual Rainfall[a]	Relative Rainfall[b]	Road Density[b]
(1) Olympic Peninsula	>240"	3	1
(2) Coast Range	60-120"	3	1
(3) Puget Trough	15-60"	2	3
(4) Northern Cascades	50-180"	3	1
(5) Southern Cascades	50-140"	3	1

Physiographic Province	Annual Rainfall[a]	Relative Rainfall[b]	Road Density[b]
(6) Okanogan Highlands	12-45"	2	2
(7) Columbia Basin	7-20"	1	2
(8) Blue Mountains	18-40"	2	1

[a] Franklin and Dyrness, 1973
[b] A rating of 3 indicates high rainfall or road density whereas ratings of 2 and 1 correspond to moderate and low values, respectively.

Table 3-12. Estimated Persistence of WSDOT Applied Herbicides in Soil.

Herbicide	Relative Application Rate[a]	Persistence[a]
2,4-D	2	1
Bromacil	2	2
Chlorsulfuron	1	1
Clopyralid	1	2
Clopyralid/2-4D	2	2
Dicamba	2	1
Dicamba/2,4-D	2	1
Dicamba/MCPA	2	1
Dichlobenil	2	2
Diuron	2	2
Fosamine (ammonium salt)	2	1
Glyphosate	2	2[c]
Metsulfuron methyl	1	3
Oryzalin	2	1
Picloram	2	3
Sulfometuron methyl	1	1
Triclopyr	2	1
Petroleum distillate	No data	1
New Herbicides		
Bromoxynil	1	1
Diflufenzopyr	1	1
Flumioxazin	1	1
Fluroxypyr	1	3
Imazapyr	2	3
Isoxaben	2	3
Norflurazon	2	3
Oxadiazon	2	3
Pendimethalin	2	3
Pyraflufen	1	3
Sulfentrazone	1	3
Tebuthiuron	2	3

a. Relative application rates defined by WSDOT; 1 = <1 lb/acre; 2 = 1 to 10 lbs/acre, 3= >10 lbs/acre.
[b] Persistence ratings are based on reported half life in soil or water as follows:
1: 0 to 3 months
2: 4 to 6 months
3: Greater than 6 months

[c] This persistence rating is for sandy loam soils; in most soils Glyphosate has a soil half life less than 60 days.

Table 3-13. Relative Exposure[a] of Aquatic Organisms to Herbicides by Physiographic Province.

Chemical	Physiographic Province[b]							
	1	2	3	4	5	6	7	8
2,4-D	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Bromacil	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Chlorsulfuron	1.5	1.5	1.8	1.5	1.5	1.5	1.3	1.3
Clopyralid	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Clopyralid/2,4-D	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Dicamba	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Dicamba/2,4-D	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Dicamba/MCPA	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Dichlobenil	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Diuron	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Fosamine	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Glyphosate	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Metsulfuron methyl	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Oryzalin	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Picloram	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Sulfometuron methyl	1.5	1.5	1.8	1.5	1.5	1.5	1.3	1.3
Triclopyr	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Petroleum distillate	1.8	1.8	2.0	1.8	1.8	1.8	1.5	1.5
Herbicides Evaluated in 2005								
Bromoxynil	1.5	1.5	1.8	1.5	1.5	1.5	1.3	1.3
Diflufenzopyr	1.5	1.5	1.8	1.5	1.5	1.5	1.3	1.3
Flumioxazin	1.5	1.5	1.8	1.5	1.5	1.5	1.3	1.3
Fluroxypyr	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Imazapyr	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Isoxaben	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Norflurazon	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Oxadiazon	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Pendimethalin	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0
Pyraflufen	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Sulfentrazone	2.0	2.0	2.3	2.0	2.0	2.0	1.8	1.8
Tebuthiuron	2.3	2.3	2.5	2.3	2.3	2.3	2.0	2.0

[a] Exposure is based upon average annual precipitation and road density in each of the physiographic provinces as well as the maximum application rate and half life of each chemical in soil. The relative exposure ratings are as follows: Exposure scale: 1-1.3: Low exposure; 1.4-1.7: Moderately low; 1.8-2.3 Moderate; 2.4-2.6: Moderately High; 2.7-3.0: High

[b] Physiographic provinces:
(1) Olympic Peninsula
(2) Coast Ranges
(3) Puget Trough
(4) Northern Cascades
(5) Southern Cascades
(6) Okanogan Highlands
(7) Columbia Basin
(8) Blue Mountains

Table 3-14. Relative Risk[a] to Fish From Exposure to Herbicides by Physiographic Province.

Chemical	Physiographic Province[b]							
	1	2	3	4	5	6	7	8
2,4-D	1	1	2	1	1	1	1	1
Bromacil	1	1	2	1	1	1	1	1
Chlorsulfuron	1	1	1	1	1	1	0	0
Clopyralid	1	1	1	1	1	1	1	1
Clopyralid/2,4-D	1	1	1	1	1	1	1	1
Dicamba	1	1	1	1	1	1	1	1
Dicamba/2,4-D	1	1	1	1	1	1	1	1
Dicamba/MCPA	1	1	1	1	1	1	1	1
Dichlobenil	2	2	2	2	2	2	2	2
Diuron	3	3	3	3	3	3	2	2
Fosamine	1	1	1	1	1	1	1	1
Glyphosate	1	1	2	1	1	1	1	1
Metsulfuron methyl	1	1	1	1	1	1	1	1
Oryzalin	2	2	2	2	2	2	2	2
Picloram	2	2	2	2	2	2	1	1
Sulfometuron methyl	1	1	1	1	1	1	1	1
Triclopyr	2	2	2	2	2	2	2	2
Petroleum distillate	1	1	2	1	1	1	1	1
Herbicides Evaluated in 2005								
Bromoxynil	3	3	3	3	3	3	2	2
Diflufenzopyr	1	1	1	1	1	1	1	1
Flumioxazin	2	2	2	2	2	2	1	1
Fluroxypyr	1	1	2	1	1	1	1	1
Imazapyr	2	2	2	2	2	2	1	1
Isoxaben	NA	NA	NA	NA	NA	NA	NA	NA
Norflurazon	2	2	3	2	2	2	2	2
Oxadiazon	3	3	3	3	3	3	3	3
Pendimethalin	3	3	3	3	3	3	3	3
Pyraflufen	2	2	2	2	2	2	1	1
Sulfentrazone	1	1	2	1	1	1	1	1
Tebuthiuron	2	2	2	2	2	2	1	1

[a] Risk is characterized based upon likelihood of exposure and the relative toxicities of the herbicides,using the most conservative estimate of toxicity (as summarized in Table 3-10). The calculation of relative risk is as follows: (exposure rating)(toxicity)/4. The relative risk ratings are as follows:
The relative risk ratings are as follows:
1: Low risk; 2: Slight risk; 3: Moderate risk; 4: High Risk; 5: Very High Risk

[b] Physiographic provinces:
(1) Olympic Peninsula
(2) Coast Ranges
(3) Puget Trough
(4) Northern Cascades
(5) Southern Cascades
(6) Okanogan Highlands
(7) Columbia Basin
(8) Blue Mountains

Table 3-15. Relative Risk[a] to Aquatic Invertebrates from Exposure to Herbicides by Physiographic Province.

Chemical	Physiographic Province[b]							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2,4-D	2	2	2	2	2	2	2	2
Bromacil	1	1	2	1	1	1	1	1
Chlorsulfuron	1	1	1	1	1	1	1	1
Clopyralid	1	1	1	1	1	1	1	1
Clopyralid/ 2,4-D	1	1	1	1	1	1	1	1
Dicamba	1	1	1	1	1	1	1	1
Dicamba/2,4-D	1	1	1	1	1	1	1	1
Dicamba/MCPA	1	1	1	1	1	1	1	1
Dichlobenil	3	3	3	3	3	3	2	2
Diuron	3	3	3	3	3	3	2	2
Fosamine (ammonium salt)	1	1	1	1	1	1	1	1
Glyphosate	1	1	2	1	1	1	1	1
Metsulfuron methyl	1	1	1	1	1	1	1	1
Oryzalin	1	1	2	1	1	1	1	1
Picloram	2	2	2	2	2	2	1	1
Sulfometuron methyl	1	1	1	1	1	1	1	1
Triclopyr	1	1	1	1	1	1	1	1
Petroleum Distillate	2	2	2	2	2	2	2	2
Herbicides Evaluated in 2005								
Bromoxynil	3	3	3	3	3	3	2	2
Diflufenzopyr	1	1	1	1	1	1	1	1
Flumioxazin	2	2	2	2	2	2	2	2
Fluroxypyr	2	2	2	2	2	2	1	1
Imazapyr	2	2	2	2	2	2	1	1
Isoxaben	NA	NA	NA	NA	NA	NA	NA	NA
Norflurazon	2	2	3	2	2	2	2	2
Oxadiazon	3	3	3	3	3	3	3	3
Pendimethalin	3	3	3	3	3	3	3	3
Pyraflufen	2	2	2	2	2	2	1	1
Sulfentrazone	1	1	2	1	1	1	1	1
Tebuthiuron	2	2	2	2	2	2	1	1

[a] Risk is characterized based upon likelihood of exposure and the relative toxicities of the herbicides using the most conservative estimate of toxicity (as tabulated in Table 3-10). The calculation of relative risk is as follows: (exposure rating)(toxicity)/4. The relative risk ratings are as follows:
1: Low risk; 2: Slight risk; 3: Moderate risk; 4: High Risk; 5: Very High Risk

[b] Physiographic provinces:
(1) Olympic Peninsula
(2) Coast Ranges
(3) Puget Trough
(4) Northern Cascades
(5) Southern Cascades
(6) Okanogan Highlands
(7) Columbia Basin
(8) Blue Mountains

Table 3-16. Range of Relative Acute Aquatic Toxicities of the Herbicides Currently Used by the WSDOT based on current literature findings and acute toxicity criteria provided by the U.S. EPA[a].

Herbicide	Toxicity[b]		Herbicide	Toxicity	
	F	I		F	I
2,4-D	1-4	2-3	Metsulfuron methyl	1	1
Bromacil	1-2	1-2	Oryzalin	3	1-4
Chlorsulfuron	1	1	Picloram	1-3	1-3
Clopyralid	1	1	Sulfometuron methyl	2	1-2
Clopyralid/2,4-D	1	1	Triclopyr	1-5	1
Dicamba, Dicamba/2,4-D, Dicamba/MCPA	1-2	1	Fosamine (ammonium salt)	1	1
Dichlobenil	2-3	3-4	Glyphosate	1-3	1-2
Diuron	3-4	3-4			
Herbicides Evaluated in 2005					
Herbicide	**Toxicity[b]**		**Herbicide Toxicity**	**Toxicity[b]**	
	F	**I**		**F**	**I**
Bromoxynil	4-5	5	Norflurazon	2-3	2-3
Diflufenzopyr	1-2	1-2	Oxadiazon	3-4	3-4
Flumioxazin	2-3	3-4	Pendimethalin	3-4	4
Fluroxypyr	1-2	1-4	Pyraflufen	1-4	1-4
Imazapyr	1-2	1-2	Sulfentrazone	1-2	2
Isoxaben	NA	NA	Tebuthiuron	1-2	1-2

[a] Relative acute toxicities of the herbicides (U.S. EPA, 1985):

LC_{50} or EC_{50} (mg/l)	Toxicity Category
<0.1	Very highly toxic (5)
0.1 to 1	Highly toxic (4)
>1 to ≤10	Moderately toxic (3)
>10 to ≤100	Slightly toxic (2)
>100	Practically non-toxic (1)

[b] F: Fish; I: Aquatic Invertebrates

6.0 Conclusions

A wide range of fish and aquatic invertebrates, both freshwater and marine, occur in the surface waters of Washington State. The types of fish, invertebrate and aquatic plant species depend upon such factors as water temperature, salinity, and flow conditions. Because of the diversity of aquatic habitats throughout the state, no single species is representative of all conditions where aquatic biota could be exposed to herbicides from WSDOT's integrated pest management program for vegetation control. A full quantitative risk assessment that evaluates the potential impacts to fish, macro invertebrates, and non-target vegetation was not possible under the current effort because site-specific data would be required. Thus, a modeling exercise was conducted to evaluate the relative risk of each of the herbicides used by integrating estimated exposure (from rainfall data and road density) and inherent toxicity of the herbicide from the most sensitive species tested. This relative risk assessment does not establish the absolute risk of any herbicide, but it is useful in comparing estimated relative risks between different herbicides to prioritize efforts for risk reduction. All estimates are subject to the uncertainties and data gaps identified in section 4.0.

Based on expected run-off patterns, road density, persistence and known toxicity, none of the herbicides currently used by the WSDOT fell into the high or very high relative aquatic risk category if applied in accordance with the methods and application rates considered in this risk assessment. Changes to WSDOT's application practices have resulted in significant reductions in aquatic risks in their use of Diuron, Picloram, Dichlobenil, and Glyphosate since the first evaluation of aquatic risk was conducted in 1993 (*i.e.*, when using the mean toxicity rating of the herbicides from the literature, as conducted previously). In this addition to the EIS, risks to aquatic organisms were estimated by calculating the product of exposure and the mean toxicity rating. When the toxicity rating only spanned 2 values, the more conservative rating was used. Even if the maximum toxicity value was used, none of the risks to aquatic organisms exceeded the moderate level.

Based on the methods described in Section 3.2, risks were calculated on a scale of 1 to 5, with 1 noted as being a low risk and 5 assigned a value of very high risk. Among the herbicides previously evaluated in 2003, only Diuron was found to present a moderate risks to fish in all physiographic provinces except for the Columbia Basin and Blue Mountains, where the risk was characterized as slight. Similarly, Diuron was found to present a moderate risk to aquatic invertebrates in the same physiographic provinces. Likewise, Dichlobenil was also found to present a moderate risk to aquatic invertebrates in all physiographic provinces except for the Columbia Basin and Blue Mountains, where the risk was characterized as slight. Among the additional 12 herbicides added in 2005, Oxadiazon and Pendimethalin were found to present moderate risks to both fish and aquatic organisms in all physiographic provinces examined. Bromoxynil presents a moderate risk to fish and aquatic invertebrates in all physiographic provinces except for the Columbia Basin and Blue Mountains. Norflurazon presents a moderate risk to both fish and aquatic organisms in the Puget Trough, but only a slight risk in the other 7 physiographic provinces.

Although results from this assessment suggest that current WSDOT practices do not present significant risks to aquatic organisms, it must be reiterated that these results represent a simplified model with several important limitations as described above. As additional data become available for these herbicides and others that are considered for use by WSDOT, a reevaluation of the potential impacts to aquatic organisms is encouraged. Important areas of uncertainty include effects from inert ingredients and adjuvants, effects to sensitive or threatened and endangered species, and potential synergism resulting from exposure to multiple chemicals. In addition, relative risks to aquatic plants could not be ascertained due to lack of toxicity data, but effects on primary producers (*e.g*., diatoms and microalgae) would not be unexpected based on limited research findings of others to date. Finally, it should be noted that spills and other extreme events were not considered in this aquatic risk assessment and the effects from such events could be significant on aquatic biota and their habitat. Revised and more precise information on road density and rainfall will also increase value of future assessments. Despite the numerous uncertainties and data gaps described in this assessment, the exposure mitigation measures outlined in Section 4.0 provides a useful framework for reducing herbicide exposure and potential risks among aquatic organisms.

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