Screening-Level Ecological Risk Assessment of the Proposed Use of the Herbicide Imazamox to Control Invasive Japanese Eelgrass (*Zostera japonica*) in Willapa Bay, Washington State

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Acronyms and Abbreviations

ac	Acre
AF	Absorption fraction
AHAS	Acetohydroxyacid synthase
ai	Active ingredient
ai/ha	Active ingredient per hectare
a.k.a.	Also known as
ALS	Acetolactate synthase
AUF	Area use factor
BASF	Badische Anilin- und Soda-Fabrik
BCF	Bioconcentration factor
BW	Body weight
°C	Degrees Celsius
CAS	Chemical Abstracts Service
CM	Contaminant concentration in media(s) of concern
cm	Centimeter
cm^2	Square centimeters
cm/hr	Centimeters per hour
COPCs	Chemicals of potential concern
CR	Contact rate
CsI	Maximum concentration in soil
CSM	Conceptual site model
DT_{50}	Time it takes for 50% of the material to degrade (Half-life)
DT_{90}	Time it takes for 90% of the material to degrade
EC_{50}	Maximal effect concentration of 50%
EC_{90}	Maximal effect concentration of 90%
EEC	Environmental exposure concentration
EIS	Environmental Impact Statement
EPA	Environmental Protection Agency
ESA	Endangered Species Act
EUP	Experimental use permit
FI	Fractional intake
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FIR	Food ingestion rate
ft	Foot or feet
g/l	Gram per liter
g/mol	Gram per mole
Ha	Hectare
HI	Hazard indices
HPLC	High-performance liquid chromatography
HQ	Hazard quotient
hr	Hour
IC_{10}	Inhibition concentration at 10%
IC_{50}	Inhibition concentration at 50%
IPM	Integrated Pest Management
Kg	Kilogram
kJ/g	Kilojoule per gram
km	Kilometer
km^2	Square kilometer
K_{ow}	Octanol-water partition coefficient
LC_{50}	Concentration at which 50% lethality occurs
LC_{90}	Concentration at which 90% lethality occurs
LD_{50}	Dose at which 50% lethality occurs
LOAEC	Lowest observable adverse effect concentration
LOAEL	Lowest observable adverse effect level
LOEC	Lowest observable effect concentration
M	Meter
m^2	Square meters
M^3	Meters cubed
mg/kg	Milligrams per kilogram
mg/l	Milligrams per liter
min	Minute
mL	Milliliters
MLLW	Mean lower low water
ml/min	Milliliters per minute
mol	Mole
mph	Miles per hour
NA	Not applicable/ available
NEPA	National Environmental Policy Act
nM	Nanometer
NOAEC	No observable adverse effect concentration
NOAEL	No observable adverse effect level
NOEC	No observable effect concentration
NPDES	National Pollutant Discharge Elimination System
OC	Organic carbon
oz/ac	Pounds of formulation per 100 gallons of spray per acre
Pa	Pascals
Pi	Fraction of diet as item i
pK_a	Acid dissociation constant
ppb	Parts per billion
ppm	Parts per million
RGR	Relative growth rate
RM	River miles

SEPA	State Environmental Policy Act
SIR	Sediment ingestion rate
SLERA	Screening level ecological risk assessment
TDI	Total daily intake
T&E	Threatened and endangered
TRV	Toxicity reference value
wk	Week
WSDA	Washington State Department of Agriculture
WSU	Washington State University
µg	Microgram
µg/cm^2	Microgram per square centimeter
µg/g	Microgram per gram
µg/kg	Microgram per kilogram
µg/l	Microgram per liter
µM	Micrometer

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1 Introduction

Washington State Department of Agriculture (WSDA) has legislative mandate to control noxious and invasive weeds in the State of Washington. Noxious weeds are defined as plants that are highly destructive, competitive, or difficult to control by cultural or chemical practices (RCW 17.10.010). Japanese eelgrass (*Zostera japonica*) is a non-native aquatic invasive species in Washington. The Washington State Noxious Weed Control Board recently registered Japanese eelgrass as a Class C noxious weed) on commercially managed, Willapa Bay shellfish beds only. This restrictive classification reflects the dual recognition of the adverse effects the species has recently created through its rapid spread onto commercial shellfish beds, and the beneficial ecological functions the non-native seagrass may provide for other managed species for which the scientific community has not reached consensus. Impacts identified with the establishment of Japanese eelgrass include changing water flow patterns, trapping sediments, altering species assemblages, disrupting foraging habitat for shorebirds, and reducing hardshell clam set and growth (Tsai et al. 2010, Patten 2008; Fisher et al. 2011).

Typical of other biogenic invasive species—whether native or non-native—these impacts may benefit some species and adversely impact others. While these outcomes of establishment and spread reflect the potential outcomes of the Japanese eelgrass introduction, authorization of herbicide treatment to control the species on clam beds, as under consideration by the Washington State Department of Ecology (Ecology) National Pollutant Discharge Elimination System (NPDES) program, may pose ecological risks to non-target organisms independent from the habitat alteration associated with establishment of Japanese eelgrass, and therefore must be evaluated before herbicide is used for Japanese eelgrass control.

This screening level ecological risk assessment (SLERA) evaluates ecological risks posed by one chemical control method currently proposed, namely the use of the herbicide imazamox (Clearcast™ formulation) to locally remove and manage the invasive Japanese eelgrass on intertidal beds used for clam culture. To evaluate the efficacy of management of Japanese eelgrass with the herbicide, a series of studies were conducted under experimental use permits by Washington State University (WSU) beginning in 2006 (Appendix A). These studies provided data needed to refine the treatment approach. Recently released draft permit language from Ecology (2012) reflect these treatment efficacy data, and have now provided the necessary information to describe the proposed treatment regime that may be authorized and implemented under the NPDES program and from which risks must be characterized. In May 2012, WSU (Patten and Haldeman 2012) collected additional empirical data that had been requested by the risk assessment team in order to better understand environmental exposure concentrations (EECs). These information sources were sufficient to proceed with finalizing this SLERA.

This SLERA does not address all aspects of environmental review that would be required under Washington's State Environmental Policy Act (SEPA). Rather, it provides information on chemical (imazamox) risk to ecological receptors from the proposed program only. While information provided herein can be used to support decision making, this document should not be considered a decision document. It does not address economic or ecological impacts of the eelgrass itself as would be considered, for example, under a no-action alternative under SEPA. It also does not assess impacts from the proposed herbicide use, as required under SEPA, on other resources typically evaluated under SEPA (e.g., cultural resources). These elements of environmental review are being addressed by Ecology in a SEPA-Environmental Impact Statement (EIS). Because there is no federal funding or authorizations that would be associated with the proposed treatment, rules

outlined in SEPA will direct the overall impact assessment in the EIS, and National Environmental Policy Act (NEPA) provisions will not apply for environmental compliance.

Risk assessment is an organizational framework for understanding and integrating information related to exposure and toxicology, in order to predict the likelihood, severity, and spatial extent of adverse effects from a stressor. Toxicology is the study of poisons. It examines and attempts to define the range in responses of an organism or organisms to variable doses of a chemical or chemicals. Thus, the most important factors regulating chemical toxicity are the exposure dose, the duration of exposure, and the potency of the chemical. The genotype, and nutritional and physiological state of a plant or animal (i.e., "ecological receptor," as used throughout this report), at the time of exposure can also affect the severity of effects. The introduction of chemicals into an ecosystem can directly harm organisms, or may indirectly affect their fitness—the ability of an animal to survive and produce viable offspring. The results of chemical exposure may be immediately apparent or may become noticeable only after considerable delay. In ecological risk assessment, the effects of chemicals are examined not only at the individual organismal level as outlined above, but also at the broader population and ecosystem level where possible and practicable. Thus, the purpose of this SLERA is to consider the nature, magnitude, and permanence of predicted effects to receptors from exposure to imazamox, based on maximum projected application rates and integrated pest management practices. The SLERA relies heavily on ecological hazard studies that have been conducted over the past several years, product registration study results, and conservative deterministic exposure modeling at the organismal level. Effects at the organismal level are presumed to be reflective of potential effects at the population level, though no quantitative measures of effect at the population level are calculated.

The outline and methods of the main body of this report generally reflect ecological risk assessment guidelines developed by the U.S. Environmental Protection Agency (EPA) (EPA 1998). The report begins with problem formulation, which summarizes the scope of the Japanese eelgrass infestation for which management is proposed, the proposed management with imazamox, and the approach to the assessment of the risks from imazamox use. The problem formulation is followed by the effects assessment, which relates the current understanding of imazamox environmental fate and its toxicity to the range of target and non-target organisms. The effects assessment is followed by the exposure assessment, in which the pathways and doses of potential imazamox exposure to ecological receptors are evaluated. Estimated or empirically measured exposures are then compared to reported toxicity thresholds from previous testing to derive hazard quotients that describe the potential adverse effects posed by the proposed herbicide treatment.

Ideally, risk assessments rely on toxicity data for the species of greatest interest to the ecosystem under study, such that risks can be characterized with minimal uncertainty. In the absence of species-specific toxicity data for all receptors, it was necessary to extrapolate toxicity data across species in certain cases. The use of surrogate species with similar dietary and/or behavior patterns (i.e., guild species) has been shown to provide a relatively reliable predictor of potential toxicity when toxicity data are lacking for specific species found in an area where a potential chemical risk exists (Sappington et al. 2001). For those cases where species-specific data of particular relevance to Willapa Bay were not available, standard EPA and other test species were used as guild surrogates to model potential exposure and risks to terrestrial and marine organisms that use the Willapa Bay system. Thus, this assessment used surrogate species such as the rat, rabbit, quail, and mallard to gauge exposure to Willapa Bay wildlife that could be exposed to the herbicide. The rat serves as a reasonable surrogate for other omnivores, the rabbit for herbivores, and the quail and mallard for upland birds and waterfowl, respectively.

The objectives of this SLERA are as follows:

- To describe reported environmental fate and transport properties of imazamox.
- To identify species that may be impacted in different regions where imazamox could be applied.
- To describe reported toxicity posed by imazamox to marine and estuarine aquatic organisms.
- To describe reported toxicity posed by imazamox to terrestrial wildlife.
- To describe reported toxicity posed by imazamox to non-target vegetation. To estimate ecological exposures associated with complete exposure pathways, taking into account environmental fate and transport. To characterize risks posed to other environmental components potentially affected by imazamox.

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2 Problem Formulation

This chapter provides an overview of the Japanese eelgrass infestation in Washington waters. Additionally, herbicide management means considered under the integrated pest management (IPM) program, methodology and assessment endpoints by which risks to ecological receptors are quantified and characterized from the proposed used of herbicide for Japanese eelgrass control under the IPM program, and the conceptual site model (CSM) used to consider exposure to relevant ecological receptors are described.

2.1 OVERVIEW OF JAPANESE EELGRASS INFESTATION IN WASHINGTON STATE

Japanese or dwarf eelgrass was presumably introduced to Washington State with Japanese oyster (*Crassostrea gigas*) spat in northern Puget Sound in the 1930s (Bulthuis et al. 2005, Mumford 2007), and subsequently observed on the Washington State coast in 1957 (Hitchcock et al. 1969 as cited in Major et al. 2004). Since its introduction, Japanese eelgrass has spread along Washington's outer coast and throughout northern and central Puget Sound. According to the Alaska Natural Heritage Program (ANHP 2005), "colonization of sparsely vegetated or bare intertidal flats by dwarf eelgrass represents a drastic modification of habitat." Thom et al. (2011) identified Japanese eelgrass to represent the primary invasive species of concern to native eelgrass, but also noted that it appeared to have relatively limited impact to native species.

2.1.1 Distribution

Japanese eelgrass is currently distributed from Vancouver Island, British Columbia to Humboldt Bay, California (McBride 2002). It generally occurs higher in the intertidal zone (0.1-1.5 m mean lower low water [MLLW]) than does native eelgrass (*Z. marina)* (0.6 m MLLW and below), colonizing open tidal mudflats and sandflats within sheltered bays and inlets of the Pacific Northwest (Ruesink et al. 2010). Aside from the development of monocultures in the mid-intertidal zone, there are also reports of mixed beds in the transition zones between the two eelgrass species (0.3 to 0.6 m MLLW), and even range extension of Japanese eelgrass into native eelgrass beds (Harrison 1982a, Thom 1987, Bulthuis et al. 2005, Ruesink et al. 2010).

As demonstrated in **Table 2-1**, Japanese eelgrass is found in multiple locations within Puget Sound, and along the Washington Coast. Grays Harbor and Willapa Bay support the greatest distribution of the grass. In Willapa Bay, Japanese eelgrass has been documented since the mid-1950s (Harrison and Bigley 1982) and until about 1998 remained relatively confined in plant density and location. Since that time, it has expanded aggressively and now carpets many areas of Willapa Bay. The anecdotal lag period observed with Japanese eelgrass in Willapa Bay may reflect time required to achieve a critical threshold size, after which, interannual variation is reduced as the population expands beyond the threshold that was initially required for establishment and growth (Almasi and Eldridge 2008). In Willapa, Japanese eelgrass also appears to colonize intertidal hillocks that are at an elevation that would not, at least, initially support native eelgrass.

Table 2-1 Distribution of Japanese Eelgrass in Puget Sound and the Washington Coast

Region	Location	County	Source
Canada-USA border	Bellingham Bay	Whatcom	Harrison and Bigley 1982
	Chuckanut Bay	Whatcom	Harrison and Bigley 1982
	East of Ferndale	Whatcom	Gaeckle et al. 2009
	Birch Bay	Whatcom	Harrison and Bigley 1982
	Semiahmoo Spit	Whatcom	Gaeckle et al. 2009
	Drayton Harbor	Whatcom	Gaeckle et al. 2009
	SE of Cherry Point	Whatcom	Gaeckle et al. 2009

Region	Location	County	Source
San Juan-Strait of Juan de Fuca	Eastsound County Park (Orcas Island)	San Juan	Gaeckle et al. 2009
	North Side of Crane Island	San Juan	Gaeckle et al. 2009
	Picnic Cove	San Juan	Gaeckle et al. 2009
North Puget Sound	Padilla Bay	Skagit	BMNHC 2006, Gaeckle et al. 2009
	Samish Bay	Skagit	Gaeckle et al. 2009
	Similk Bay	Skagit	Gaeckle et al. 2009
	North Possession	Island	Gaeckle et al. 2009
	Useless Bay (Whidbey Island)	Island	Gaeckle et al. 2009
	Ebey's Slough	Snohomish	BMNHC 2006
	Hat Slough	Snohomish	BMNHC 2006
	Jetty Island	Snohomish	BMNHC 2006
	Tulalip Bay	Snohomish	Gaeckle et al. 2009
	Snohomish Delta N	Snohomish	Gaeckle et al. 2009
	Edgewater, Possession Sound	Snohomish	Gaeckle et al. 2009
	Kilisut Harbor	Jefferson	ENVIRON 2009
Hood Canal	Oak Bay	Jefferson	Gaeckle et al. 2009
	S. of Tala Point	Jefferson	Gaeckle et al. 2009
	E. of Squamish Harbor	Jefferson	Gaeckle et al. 2009
	N. of Thorndyke Bay	Jefferson	ENVIRON 2009, Gaeckle et al. 2009
	Dabob Bay	Jefferson	Gaeckle et al. 2009
	S. of Long Spit	Jefferson	Gaeckle et al. 2009
	Quilcene Bay	Jefferson	Gaeckle et al. 2009, USFWS 2009a
	Toanados Peninsula	Jefferson	Gaeckle et al. 2009
	Dosewallips	Jefferson	Gaeckle et al. 2009
	N of Port Gamble	Kitsap	Gaeckle et al. 2009
	Warrenville	Kitsap	Gaeckle et al. 2009
	Anderson Cove	Kitsap	Gaeckle et al. 2009
	Stimson Creek	Mason	Gaeckle et al. 2009
	Annas Bay	Mason	USFWS 2009a
	Lynch Cove	Mason	Gaeckle et al. 2009
	Forest Beach	Mason	Gaeckle et al. 2009
Central Puget Sound	Sinclair Inlet	Kitsap	USFWS 2009a
	Agate Pass Bridge SE (Bainbridge Island)	Kitsap	Gaeckle et al. 2009
	Murden Cove (Bainbridge Island)	Kitsap	Gaeckle et al. 2009
	Quartermaster Harbor	King	BMNHC 2006
	Tramp Harbor (Vashon Island)	King	Gaeckle et al. 2009
	Paradise Cove (Vashon Island)	King	Gaeckle et al. 2009
	Poverty Bay	King	Gaeckle et al. 2009
	Dumas Bay	King	Gaeckle et al. 2009
	Piner Point (Maury Island)	King	Gaeckle et al. 2009
South Puget Sound	North Bay, Case Inlet	Mason	USFWS 2009a
	Taylor Bay, Case Inlet	Mason	ENVIRON 2009
	Harstine Island, Case Inlet	Mason	ENVIRON 2010
	Totten Inlet	Thurston	ENVIRON 2009
	Burley Spit, Carr Inlet	Pierce	Gaeckle et al. 2009
Washington Coast	Willapa Bay	Pacific	Harrison and Bigley 1982, BMNHC 2006
	Grays Harbor	Grays Harbor	Harrison and Bigley 1982, BMNHC 2006

2.1.2 Biology

Japanese eelgrass is an annual plant with high seed production (Ruesink et al. 2010). However, in Willapa Bay and other Pacific Northwest estuaries, it behaves like a perennial (Ruesink et al. 2010). Germination typically occurs in the spring (May to September) due to light and temperature triggers (Harrison 1982a). Ruesink et al. (2010) reported a wider sexual reproductive season in Willapa Bay, with seed recruitment peaking in March (up to 57%) and continued until June (seed recruitment), and flowering began in June and continued through October. In Boundary Bay, vegetative shoots are typically more dominant in the lower intertidal zone in May, but by the end of September the reverse is true (Harrison 1982a). Additionally, in Willapa Bay, the average shoot growth of Japanese eelgrass increases dramatically from May to July due to production from seed recruitment (Ruesink et al. 2010). A smaller increase occurs from late June to August/September, which is when production via flowering shoots occurs. There is a long season of asexual reproduction (branching) in Willapa Bay from October through December.

Eelgrass provides many key ecological functions within estuarine and marine systems, including primary production of organic carbon, buffering wave energy and tidal current, trapping sediments, and stabilizing substrate (Lacy 2004). Retention of organic matter and sediment contributes to the productivity of nearshore environments. Although these functions are provided by Japanese eelgrass, they occur with habitat modification through the conversion of bare mudflat communities to vegetated communities (Merrill 1995, Ruesink et al. 2010). Tsai et al. (2010) reported a reduction in water flow up to 40% by Japanese eelgrass introduced into mudflats of Willapa Bay.

Native eelgrass may compete for space with Japanese eelgrass at the lower intertidal zones where the two species overlap; depending on site-specific conditions, the spread of native eelgrass into shallower habitat may also be facilitated by Japanese eelgrass (Fisher et al. 2011). Merrill (1995) reported that Japanese eelgrass inhibited leaf growth and shoot recruitment of native eelgrass in August within Padilla Bay. In a separate study, Hourdequin (1994) found that native eelgrass grew significantly faster in areas isolated from Japanese eelgrass. Harrison (1982b) reported that under simulated spring conditions (9ºC, 12 hr light: 12 hr dark, low irradiance), Japanese eelgrass could compete successfully with native eelgrass when both were submerged continuously. In contrast, under simulated summer conditions (18ºC, 14 hr light: 10 hr dark, higher irradiance), the vegetative growth of native eelgrass was more than twice that of Japanese eelgrass. Finally, Ruesink et al. (2010) reported that the two eelgrass species had similar patterns of productivity in Willapa Bay, although Japanese eelgrass significantly outperformed native eelgrass in flowering and seed germination. Conversely, native eelgrass was shown to negatively affect the lower range of Japanese eelgrass. The ability of Japanese eelgrass to rapidly colonize reflects its broad environmental tolerances to seasonal and tidal variation. While its ability to replace or facilitate the spread of native eelgrass remains unclear, experimental treatment of intertidal beds discussed later in this report has shown that Japanese eelgrass recolonizes beds to pre-control densities within two years following treatment.

2.2 ECOLOGICAL RECEPTORS, COMMUNITY DESCRIPTIONS, AND THREATENED AND ENDANGERED SPECIES IN WILLAPA BAY

The Willapa Bay ecosystem supports several priority species, some of which are listed as threatened or endangered (T&E) under the Endangered Species Act (ESA). An overview of the ecological communities and ecological receptors in Willapa Bay is provided below, as this is the only area where herbicide treatment to control the grass is proposed under Ecology's draft permit language (Ecology 2012).

2.2.1 Physical Description of Willapa Bay

Willapa Bay is located in the southwestern corner of the Washington coast (**Figure 2-1**). It is Washington's largest outer coast estuary, and is approximately 38 km long and 8 km wide (Gringas et al. 2000, Cohen et al. 2001). Willapa Bay is almost fully enclosed by the Long Beach Peninsula, a 30 km-long barrier spit that was formed by the deposition of Columbia River sediments. At high tide, the aquatic environment is approximately 88,000 acres, and the total tideland area is approximately 45,000 acres (Gringas et al. 2000, WGHOGA 2006). The combination of relatively small size and extensive tidelands contribute to substantial tidal exchange, which is estimated at nearly half its volume during a single spring tide (Cohen et al. 2001, Dumbauld et al. 2009).

Freshwater input follows a Mediterranean climate pattern, in that the input is low during summer and high in winter. Based on data collected by Western Regional Climate Center (WRCC 2009), Willapa Basin at the Long Beach Experimental Station (454748) received an average of 79.9 inches of rain per year over the past 42 years of record (1967-2009). Riverine input is also a significant influence on circulation and water exchange. Willapa Bay has a drainage basin of approximately 1,865 square kilometers (km^2), with a total of nine rivers and several sloughs that drain into the bay (Jennings et al. 2003). The main tributaries of Willapa Bay include the North, Willapa, and Naselle Rivers. The Palix River is a minor contributor to the mean daily runoff. Mean daily runoff to Willapa Bay represents approximately <0.05% of the bays total volume.

2.2.2 Biology of Willapa Bay

The Willapa Basin supports an ecosystem largely unaltered by urban development; however, it has been significantly impacted by the colonization of non-indigenous exotic species. Of the 892 vascular plant species in the Willapa Basin (which includes headwater habitat outside of the brackish estuary), approximately 250 species have been introduced. Similarly, 30 of the 473 species of vertebrates identified in the basin have been introduced (Cohen et al. 2001). Approximately 34 exotic aquatic plant and animal species were identified within the Willapa Bay estuary during a 2000 research expedition sponsored by the Washington State Department of Natural Resources (WDNR) Nearshore Habitat Program (Cohen et al. 2001). Within the estuary habitat of the basin, the two most significant plant species introduced are Japanese eelgrass and smooth cordgrass (*Spartina alterniflora)*.

2.2.2.1 Eelgrass Habitat

Willapa Bay contains extensive eelgrass habitat. Mapping efforts of the extent of Japanese eelgrass have been addressed by Ruesink et al. (2006). There was approximately 6,768 acres of Japanese eelgrass in Willapa Bay in 2004, which was equivalent to 7.7% of Willapa Bay's total area (88,217 acres). This was compared to native eelgrass, which occupied approximately 9.6% of Willapa Bay in 2004.

Interactions between native eelgrass and Japanese eelgrass were discussed previously in **Section 2.1.2**. Although it is possible that each species negatively impacts the growth of the other, it is unclear whether Japanese eelgrass will ultimately expand farther. However, it is known that Japanese eelgrass colonization substantially decreases flow and recruits fine sediment and detritus into the mid-intertidal zone, which then displaces some benthic invertebrates, shorebirds, and other species dependent on bare mudflat habitat (Harrison 1982b, Harrison 1987, Posey 1988, Lee et al. 2001, Fisher et al. 2011). The general trend noted by Grosholz and Ruiz (2009) was a reduction in larger, surface feeding taxa and concurrent increases in smaller, subsurface detritivores. The authors hypothesized that such global shifts in the benthic community would have potentially negative impacts for higher trophic level consumers including crabs, fishes, and birds.

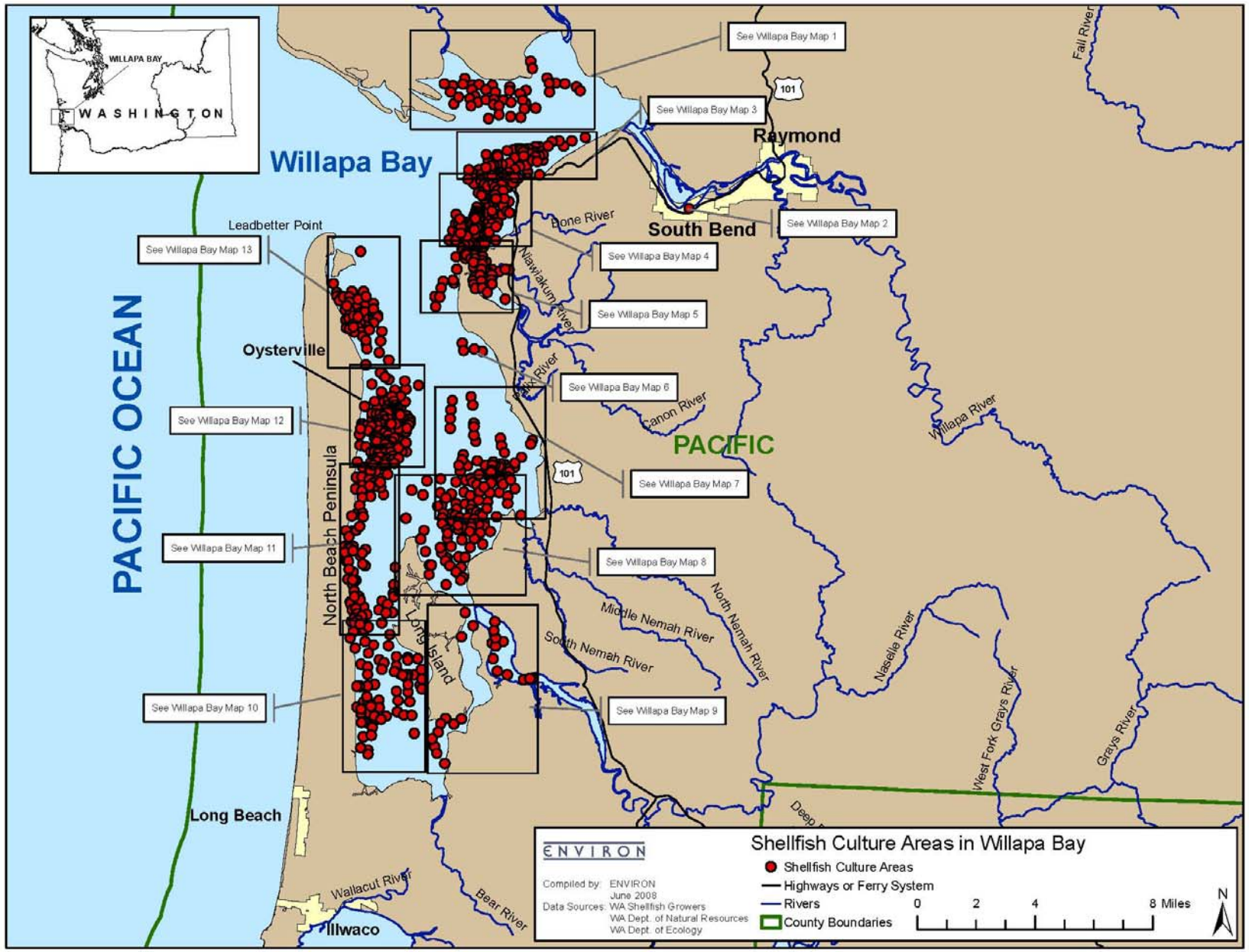


Figure 2-1 **Willapa Bay Shellfish Aquaculture Farm Locations Based on NWP 48 reporting to the US Army Corps of Engineers.**

2.2.2.2 Non-native Species

In addition to the conditions created by the introduction of Japanese eelgrass, numerous aquatic invertebrate animal species have been introduced intentionally or inadvertently into Willapa Bay during the past century. The degree to which these introductions have displaced native species is not well understood. Some of the known non-native invertebrate introductions are tabulated in **Table 2-2**.

Table 2-2 Common Non-native Invertebrates in Willapa Bay

General Taxon	Species	First Washington Record
Annelida (polychaeta)	*Hobsonia florida*	1940
	Polydora cornuta	1932
	Pseudopolydora bassarginensis	2000
	Pseudopolydora kempi japonica	1951
	Streblospio benedicti	1932
Molluska (prosobranchia)	*Crepidula fornicata*	1905
	Illyanassa obsoleta	1907
	Ocinebrellus inornatus	1924
	Urosalpinx inornatus	1890
	Nuttalia obscurata	2002 (coastline)
Molluska (bivalve)	*Crassostrea gigas*	1875
	Mya arenaria	1874
	Neotrapezium liratum	1924
	Petricolaria pholadiformis	1927
	Venerupis philippinarum	1924
Anthropoda (crustacea)	*Carcinus maenas*	1998
	Eusariella zostericola	1953
	Balanus improvisus	1853
	Nippoleucon hinumensis	1979
	Limnoria tripunctata	1871 or 1875
	Ampithoe valida	1941
	Corophium acherusicum	1905
	Corophium insidiosum	1915
	Grandidierella japonica	1966
	Jassa marmorata	1938
	Melita nitida	1938
Entoprocta (bryozoa)	*Bowerbanki gracilis*	1923
Urochordata (ascidiacea)	*Botrylloides violaceus*	1973
	Botryllus schlosseri	1944-47
	Molgula manhattensis	1949
Porifera	*Clathria prolifera*	1945-49
Cnidaria (hydrozoa)	*Cordylophora caspia*	1920
Cnidaria (anthozoa)	*Diadumene lineata*	1906

Source: Cohen et al. 2001, WDFW 2009

2.2.2.3 Commercial Shellfish

Several of the resident shellfish species in Willapa Bay support substantial commercial harvest and/or farming industries (**Table 2-3**). The most significant species include the non-native Pacific oyster (*Crassostrea gigas*) and the native Dungeness crab (PNCERS 1998). According to Nationwide Permit 48 applications to the Corps (NMFS 2009, USFWS 2009a), approximately 29% of Willapa Bay is utilized for shellfish aquaculture (37,632 acres) (**Figure 2-1**). Based on a comparison of the present and historic bivalve densities, the biomass of the Pacific oyster is 2.5

times greater than what the native oyster (*Ostrea lurida*) used to be in Willapa Bay (Ruesink et al. 2005). However, the authors noted that the difference in filtration rate is likely less because the native oyster has a higher mass-specific filtration rate.

Table 2-3 Commercial Shellfish Species within Willapa Bay

General Taxon	Common Name	Scientific Name	Type of Species	Type of Production	
				Wild	Cultured
Molluska (bivalve)	Pacific oyster	*Crassostrea gigas*	Oyster		X
	Kumamoto	*Crassostrea sikamea*	Oyster		X
	Geoduck	*Panopea abrupta*	Clam	X	
	Quahog (hardshell)	*Arctica islandica*	Clam	X	
	Softshell clam	*Mya arenaria*	Clam	X	
	Native littleneck	*Protothaca staminea*	Clam	X	
	Manila clam	*Ruditapes philippinarum*	Clam		X
	Cherrystone	*Mercenaria mercenaria*	Clam	X	
Anthropoda (crustacea)	Dungeness crab	*Cancer magister*	Crab	X	

Source: PNCERS 1998, PCSGA 2010

2.2.2.4 Fish Species

Anadromous salmonids use Willapa Bay's major tributaries for migration, spawning, incubation and early-rearing (**Table 2-4**). Although there are no Endangered Species Act (ESA)-listed salmon or steelhead runs originating from the rivers and streams flowing into Willapa Bay (NMFS 2009), bull trout have been observed in the past in Willapa River. The closest bull trout core area to Willapa Bay is Grays Harbor (USFWS 2004). Habitat within Willapa Bay is also important habitat for larval and juvenile marine and anadromous fish rearing. It is, "arguably the most important nursery estuary on the coast for juvenile English sole" (B. Dumbauld, pers. comm., 2000). Salmon and white sturgeon have supported, and continue to support, commercial fisheries in the bay. In 2009, there were a total of 6,471 Chinook, 72,779 coho, 4,568 chum, and 179 white sturgeon landed by non-Indian commercial gillnet fisheries in Willapa Bay (WDFW 2010).

Table 2-4 Anadromous Salmonid Distribution and Utilization within Willapa Bay Tributaries

River	Species	Run	Primary Use	River Miles (RM) Used	% of Stream Used
North River (61.42 RM)	Chinook salmon	Fall	Migration only	0.08-0.70	1.0%
			Rearing and migration	0.70-5.77	8.3%
			Rearing and migration	5.77-53.42	77.6%
			Rearing and migration	53.42-57.74	7.0%
			Spawning and rearing	57.74-61.42	6.0%
	Coho salmon	N/A	Migration only	0.08-0.69	1.0%
			Rearing and migration	0.69-5.77	8.3%
			Rearing and migration	5.77-27.91	36.0%
			Spawning and rearing	27.91-53.42	41.5%
			Spawning and rearing	53.42-61.42	13.0%
	Steelhead	Winter	Migration only	0.08-0.74	1.1%
			Rearing and migration	0.74-5.77	8.2%
			Rearing and migration	5.77-53.42	77.6%
			Rearing and migration	53.42-57.73	7.0%
			Spawning and rearing	57.73-61.42	6.0%

River	Species	Run	Primary Use	River Miles (RM) Used	% of Stream Used
	Chum salmon	Fall	Migration only	0.08-0.78	1.1%
			Rearing and migration	0.78-5.77	8.1%
			Rearing and migration	5.77-53.42	77.6%
			Rearing and migration	53.42-57.96	7.4%
			Migration only	57.96-61.42	5.6%
Naselle River (37.70 RM)	Chinook salmon	Fall	Migration only	0.00-8.08	21.4%
			Rearing and migration	8.08-13.88	15.4%
			Spawning and rearing	13.88-30.64	44.5%
			Migration only	30.64-31.42	2.1%
	Coho salmon	N/A	Migration only	0.00-8.06	21.4%
			Rearing and migration	8.06-12.99	13.1%
			Spawning and rearing	12.99-31.97	50.3%
			Rearing and migration	31.97-36.57	12.2%
	Steelhead	Winter	Migration only	0.00-8.08	21.4%
			Rearing and migration	8.08-12.70	12.2%
			Spawning and rearing	12.70-31.93	51.0%
			Rearing and migration	31.93-36.57	12.3%
	Chum salmon	Fall	Migration only	0.00-8.14	21.6%
			Rearing and migration	8.14-10.61	6.5%
			Spawning and rearing	10.61-27.06	43.6%
			Migration only	27.06-29.62	6.8%
Willapa River (47.92 RM)	Chinook salmon	Fall	Migration only	0.00-5.52	11.5%
			Rearing and migration	5.52-17.59	25.2%
			Spawning and rearing	17.59-44.42	56.0%
	Coho salmon	N/A	Migration only	0.00-5.51	11.5%
			Rearing and migration	5.51-38.26	68.4%
			Spawning and rearing	38.26-46.95	18.1%
	Steelhead	Winter	Migration only	0.00-5.66	11.8%
			Rearing and migration	5.66-27.86	46.3%
			Spawning and rearing	27.86-43.09	31.8%
			Rearing and migration	43.09-44.53	3.0%
	Chum salmon	Fall	Migration only	0.00-5.78	12.1%
	Chum salmon	Fall	Rearing and migration	5.78-27.96	46.3%
			Spawning and rearing	27.96-31.69	7.8%
			Rearing and migration	31.69-36.16	9.3%
			Migration only	36.16-41.02	10.1%
			Spawning and rearing	41.02-41.35	0.7%
	Bull Trout	N/A	Migration only	0.00-31.90	66.6%
North Nemah River (14.50 RM)	Chinook salmon	Fall	Migration only	0.00-0.84	5.8%
			Rearing and migration	0.84-3.00	14.9%
			Spawning and rearing	3.00-11.54	58.9%
	Coho salmon	N/A	Migration only	0.00-0.84	5.8%
			Rearing and migration	0.84-3.20	16.3%
			Spawning and rearing	3.20-12.43	63.6%
			Rearing and migration	12.43-13.73	9.0%
			Migration only	13.73-14.09	2.5%

River	Species	Run	Primary Use	River Miles (RM) Used	% of Stream Used
	Steelhead	Winter	Migration only	0.00-0.84	5.8%
			Rearing and migration	0.84-1.84	6.9%
			Spawning and rearing	1.84-11.98	69.9%
			Rearing and migration	11.98-13.73	12.1%
	Chum salmon	Fall	Migration only	0.00-0.90	6.2%
			Rearing and migration	0.90-3.85	20.3%
			Spawning and rearing	3.85-6.42	17.8%
			Rearing and migration	6.42-7.87	10.0%
			Migration only	7.87-11.27	23.5%
South Nemah River (10.99 RM)	Chinook salmon	Fall	Migration only	0.00-2.25	20.5%
			Rearing and migration	2.25-9.19	63.2%
	Coho salmon	N/A	Migration only	0.00-2.29	20.8%
			Rearing and migration	2.29-5.55	29.6%
			Spawning and rearing	5.55-9.69	37.7%
	Steelhead	Winter	Migration only	0.00-2.24	20.4%
			Rearing and migration	2.24-8.60	57.9%
			Spawning and rearing	8.60-8.95	3.2%
			Rearing and migration	8.95-9.69	6.7%
	Chum salmon	Fall	Migration only	0.00-2.29	20.9%
			Rearing and migration	2.29-4.62	21.2%
			Spawning and rearing	4.62-5.30	6.2%
			Rearing and migration	5.30-7.34	18.6%
			Spawning and rearing	7.34-8.55	11.0%
			Rearing and migration	8.55-8.84	2.6%
			Migration only	8.84-9.48	5.9%
Bear River (8.10 RM)	Chinook salmon	Fall	Migration only	0.00-2.98	36.8%
			Rearing and migration	2.98-4.31	16.5%
	Coho salmon	N/A	Migration only	0.00-5.26	64.9%
	Steelhead	Winter	Migration only	0.00-3.00	37.0%
			Rearing and migration	3.00-4.34	16.6%
			Spawning and rearing	4.34-4.99	7.9%
	Chum salmon	Fall	Migration only	0.00-3.01	37.1%
			Rearing and migration	3.01-4.31	16.1%
			Migration only	4.31-5.26	11.7%
Niawiakum River (5.87 RM)	Coho salmon	N/A	Migration only	0.00-4.06	69.2%
			Rearing and migration	4.06-4.34	4.8%
			Spawning and rearing	4.34-4.90	9.4%
	Steelhead	Winter	Migration only	0.00-4.90	83.4%

Note: Although there are many other tributaries and sloughs that drain into Willapa Bay, the criteria for reporting tributaries were (1) the water body had to be > 10 RM and (2) it had to be identified as more than a migration corridor.
Source: StreamNet 2009

Typical estuarine species found in Washington's marine waters also utilize Willapa Bay's habitats. Arguably, the most important of these species in providing forage for Pacific salmonids, is the Pacific herring. Pacific herring spawn on the eelgrass beds in February and

March (Stick and Lindquist 2009). Stick and Lindquist (2009) commented that little is known of coastal herring stocks, but that they are likely components of large summer herring aggregations that concentrate in coastal offshore areas such as the Strait of Juan de Fuca and the west coast of Vancouver Island. The cumulative spawning biomass for coastal habitats has ranged from 0 to 694 tons annually. Documented spawning grounds for Pacific herring occur along the inner shoreline of the North Beach (Stick and Lindquist 2009). WDFW field reports between 2000 and 2003 documented herring eggs attached to Japanese eelgrass in Stackpole Harbor along the eastern shore of the Long Beach peninsula (WDFW, unpublished data), indicating the species can also provide spawning substrate for Pacific Herring .

2.2.2.5 Bird Species

A total of 115 bird species have been reported from Pacific County, primarily associated with Willapa Bay. The bay is a major migration stopover location for shorebirds in the spring and winter (ENTRIX 2003). Although rare, the ESA-listed western snowy plover (*Charadrius alexandrinus*) is known to utilize Willapa Bay habitat (BMNHC 2009). In total, an estimated 100,000 to 1,000,000 shorebirds stop to feed in the mudflats of Willapa Bay and other coastal regions of Washington State during the spring. Spring and winter peak shorebird numbers declined precipitously in the 1990's as *Spartina* had infested tidal mudflats and displaced their use of formerly available habitat (Jaques 2002, Patten and O'Casey 2007); subsequent *Spartina* control with the herbicide imazapyr over the past decade has been correlated with a recent increase in shorebird counts. As discussed by Grosholtz et al. (2009), there may be similar implications of Japanese eelgrass invasion as seen in estuaries with *Spartina*, with the distinct difference that the uptake of Japanese eelgrass (based on $\delta^{15}N$ labeled detritus) was significantly greater by benthic consumers.

Willapa Bay also provides foraging habitat for the ESA-listed marbled murrelet (*Brachyramphus marmoratus*) (USFWS 2009a), and the recently de-listed (74 FR 59443) brown pelican (*Pelecanus occidentalis*). Brown pelicans' diet consists almost entirely of fish, with their primary prey anchovy (Schreiber and Clapp 1987), and movements in the summer and fall along the coast and into estuaries can largely be tied to their prey migrations. Aerial surveys along the Washington coast have documented a yearly increase of pelicans from 922 observed in 1987 to 7,610 observed in 1991 (Jaques 1994). In contrast, the Northwest Forest Plan Effectiveness Monitoring Program (Raphael et al. 2007) documented an average density of less than one marbled murrelet/km^2 along the outer margin of Willapa Bay. According to McShane et al. (2004) the closest nesting site to Willapa Bay would be along the northwestern Olympic Peninsula on the west slope of the Olympic Mountains.

Willapa Bay is a particularly important habitat for migratory waterfowl. The distribution of ducks within Willapa Bay was modeled by Willapa National Wildlife Refuge (ENTRIX 2003). The hierarchy of distribution within Willapa Bay according to mid-winter aerial waterfowl surveys is: South Bay (47.1%) > East Bay (28.6%) > North Bay (18.8%) > West Bay (4.2%) > Peninsula (1.2%). In Boundary Bay, it was documented that migratory waterfowl grazed preferentially on Japanese eelgrass vs. native eelgrass, and even made up a significant portion of the esophagus contents of brant (57.2%), American wigeon (84.8%), and mallard (72.3%) (Baldwin and Lovvorn 1994). Brant geese (*Branta bernicla*) peak in abundance in Willapa Bay in the spring at approximately 6,900 birds (Moore et al. 2004). Goose density, in general, is positively correlated with eelgrass coverage, the proximity of other estuaries along the coast, and foraging dynamics (Baldwin and Lovvorn 1994, Wilson and Atkinson 1995, Moore et al. 2004, Moore and Black 2006). Willapa Bay waterfowl were also recently found to consume Japanese eelgrass, but not nearly to the extent identified in Boundary Bay (Appendix B). This may be a reflection of

the timing of principal use of the bay by waterfowl in the fall and winter, when Japanese eelgrass has largely senesced, rather than a preference for Japanese eelgrass over the native species.

Willapa Bay, its surrounding wildlife refuge, and the extensive contiguous lowland forests also support a diverse assemblage of terrestrial and amphibious wildlife. Some 79 species of mammals (including whales and dolphins) and 21 herptiles (reptiles and amphibians) have been reported from Pacific County, 10 of which were introduced from the east coast and other areas. Common mammal and herptile species found in Pacific County are summarized in Appendix E.

2.3 PROJECT DESCRIPTION OF HERBICIDE TREATMENT PROPOSED

Ecology proposes to issue NPDES permit coverage for the discharge of the aquatic herbicide imazamox and marker dyes into Willapa Bay from treatment of commercial clam beds (excluding geoduck culture) where these products may enter the surface waters of the State of Washington (Ecology 2012). The permit would likely cover all areas in Willapa Bay, except federal or tribal lands in the estuary. Although portions of the draft permit aided in the development of the SLERA and efficacy studies, specific requirements have not been provided by Ecology. It is certain that Ecology will require monitoring, mitigation, and restrictions to use as part of the final NPDES permit.

The treatment method authorized will likely allow application by certified applicators using ground-based boom; no aerial spraying will be authorized. The formulation considered for use is registered as Clearcast, an aquatic formulation of imazamox. The application rate of the formulation considered in this risk assessment is 16 oz/ac of imazamox (0.14 kg active ingredient/ha). The Clearcast formulation would be diluted in water and applied at 10 to 25 gallons per acre to achieve the stated active ingredient (a.e.) concentration. Applications would occur at low tide, with a minimum of 1-hour drying time. This application rate yields an undiluted theoretical application rate over non-submerged tidelands of 1.4 µg imazamox/cm^2.

No adjuvants or surfactants are proposed for use in conjunction with the use of the Clearcast imazamox formulation. Tidal elevations to which direct application would be anticipated range from approximately 0 MLLW to +4 MLLW, where clam cultivation is practiced in Willapa Bay. However, Japanese eelgrass grows at substantially higher intertidal elevations in some locations in the bay.

Details regarding the chemical properties of imazamox, its recognized toxicity, environmental fate, and environmental transport are discussed in **Section 3**. Further details regarding exposure calculations are provided in **Section 4**.

2.4 CONCEPTUAL SITE MODEL

A CSM is a narrative and/or pictorial description linking the source(s) of one or more stressor, migration pathways in varying environmental media, and the exposure pathways through which ecological receptors contact those stressors. A ‘complete exposure pathway’ simply references the opportunity for a species to be directly or indirectly exposed to the chemical agent through either dermal/contact, inhalation, or dietary (food or water ingestion) exposure. To complete an exposure pathway, there must be spatial overlap of the species to directly exposed environmental media in the receptor’s habitat (e.g., water, sediment, vegetation), or by foraging in areas either directly or indirectly exposed to treatment (e.g., eelgrass or algae, benthic invertebrates). An exposure pathway is considered incomplete if there is no plausible opportunity for contact between the receptor and the stressor (e.g., if the receptor never

occupies the habitat where the stressor is located, or occupies it only when exposure is not possible, given the environmental fate properties of the chemical). An exposure pathway is considered potentially complete but insignificant if the available evidence on the life history of the animal coupled with the treatment method proposed would be extremely unlikely to deliver exposure of biological significance. For example, a shorebird present along the same beach being treated by a sprayer might have the *potential* for inhalation exposure from inadvertent aerial drift, but the likelihood of significant exposure could be argued to be so remote as to be insignificant because the simple disturbance of the application method would cause the birds to move outside the radius of any significant aerial drift resulting from application.

Based on the proposed treatment as outlined in **Section 2.3**, CSMs were developed to illustrate the potential for imazamox exposure to terrestrial (**Figure 2-2**) and marine receptors (**Figure 2-3**) resulting from Japanese eelgrass treatment. These CSMs summarize the primary sources, pathways, and routes of exposure to the different trophic levels and ecological receptors. Exposure of ecological receptors to imazamox used to control Japanese eelgrass may occur directly or inadvertently (indirectly) through ingestion of contaminated food, water, or sediment, through inhalation of aerosol, or through direct contact (e.g., epibenthic invertebrates).

2.4.1 Specific Receptors Examined for Exposure

Plants typical to the environments where imazamox could be used to control Japanese eelgrass include native eelgrass and a variety of algal species, such as sea lettuce (*Ulva* spp.*)*. Animals may include herbivores (e.g., deer, elk and rabbit), omnivores (e.g., raccoons), terrestrial carnivores (e.g., bobcat and coyote), piscivorous and omnivorous birds (e.g., osprey, eagles and gulls), reptiles (e.g., turtles), amphibians (e.g., frogs), and insects (e.g., mosquitoes). The degree to which these primarily terrestrial animals would be exposed to imazamox largely depends on the degree to which they forage in the treated intertidal habitat at low tide.

Obligate aquatic animal species include the array of Pacific salmonids native to Washington's waters, such as coho and Chinook salmon, but also other fish species such as juvenile flatfish (Pleuronectidae), juvenile sturgeon (Acipenseridae), and bullhead (Cottidae). Additional aquatic species potentially exposed to imazamox include the vast list of benthic and epibenthic macroinvertebrates common to the intertidal zone of Washington's estuaries such as Dungeness and rock crab (*Cancer* spp.). Rather than modeling exposure to all of these (and other) plants and animals that could be exposed to imazamox from Japanese eelgrass treatment, we focused on representative receptor guilds.

Receptor guilds include species with similar life histories and niches in the environment. They are used in this SLERA as conservative surrogates for the full range of species potentially exposed to imazamox. The use of receptors implies that the general characteristics of each guild will provide risk estimates that are representative of the entire guild. As such, each guild can be extrapolated more broadly than single species estimates. For example, many species of heron and egret feed on small fish and invertebrates and require trees for roosts. As such, herons and egrets display similar life histories and would be anticipated to have similar exposures to imazamox. A single surrogate, such as the great blue heron, for which reliable life-history information is available, may be used for calculating risk and the results may then be extrapolated to the guild as a whole. This approach allows the SLERA to directly evaluate species for which the best exposure information is available and allows results to be extrapolated to a broader range of potential receptors, thereby maximizing data usage and applicability of results.

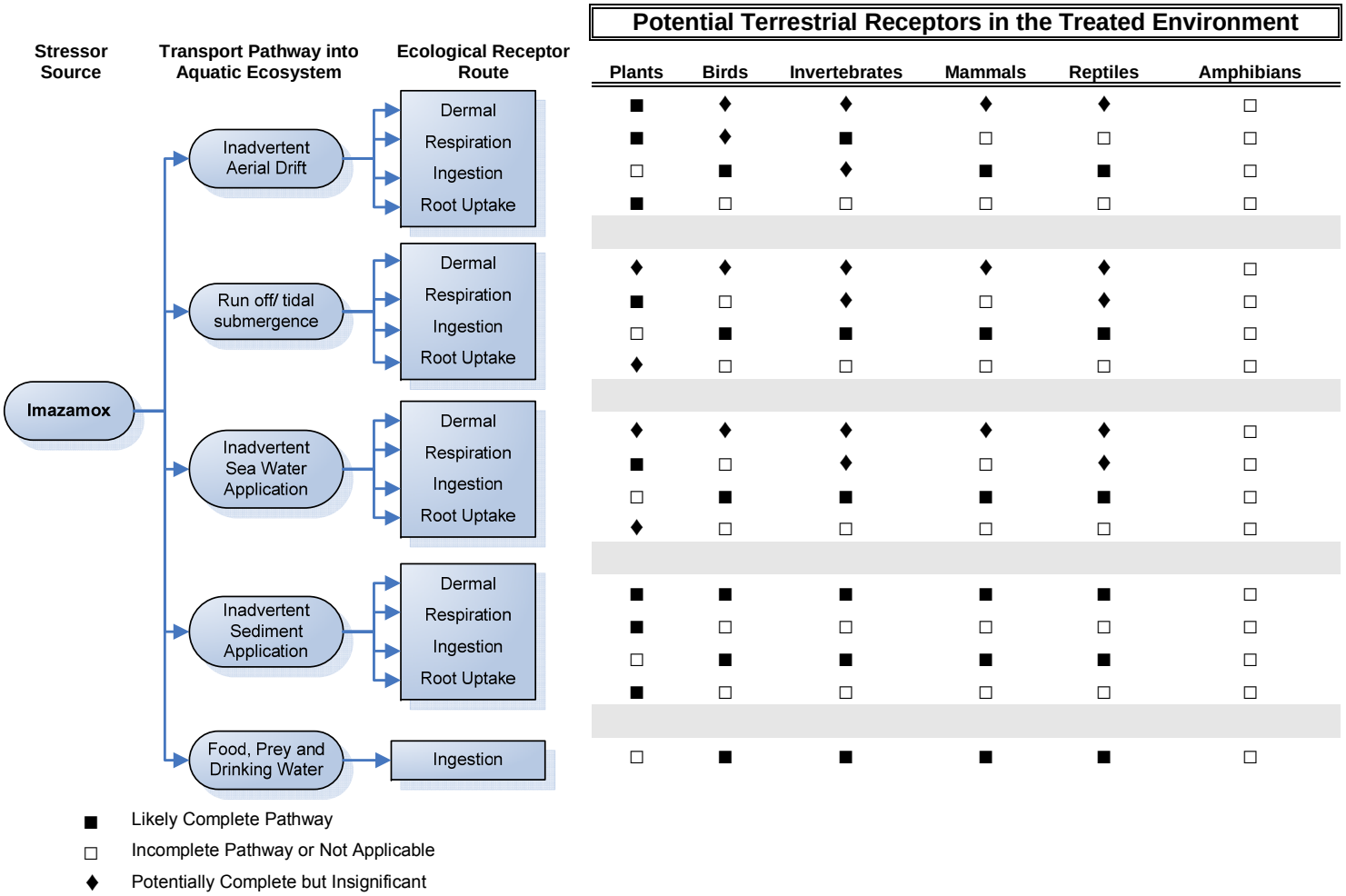
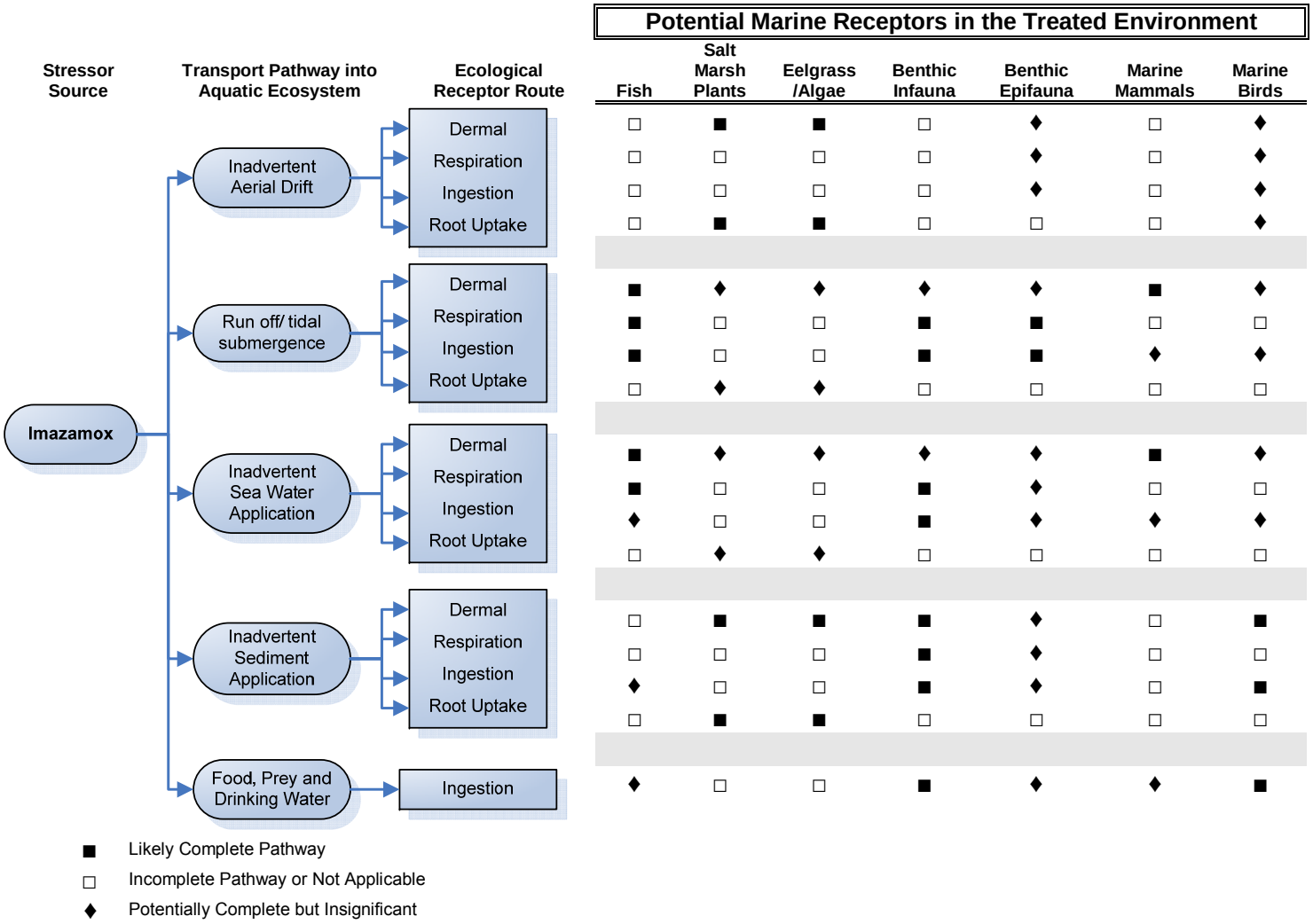


Figure 2-2 Conceptual Site Model for Imazamox Stressor Impacts to Terrestrial Receptors



Potential Marine Receptors in the Treated Environment

Fish	Salt Marsh Plants	Eelgrass /Algae	Benthic Infauna	Benthic Epifauna	Marine Mammals	Marine Birds
□	■	■	□	♦	□	♦
□	□	□	□	♦	□	♦
□	□	□	□	♦	□	♦
□	■	■	□	□	□	♦
■	♦	♦	♦	♦	■	♦
■	□	□	■	■	□	□
■	□	□	■	■	♦	♦
□	♦	♦	□	□	□	□
■	♦	♦	♦	♦	■	♦
■	□	□	■	♦	□	□
♦	□	□	■	♦	♦	♦
□	♦	♦	□	□	□	□
□	■	■	■	♦	□	■
□	□	□	■	♦	□	□
♦	□	□	■	♦	□	■
□	■	■	□	□	□	□
♦	□	□	■	♦	♦	■

Figure 2-3 Conceptual Site Model for Imazamox Stressor Impact to Marine Receptors

Surrogate species were selected in each identified receptor guild. The selected surrogates have been studied sufficiently to enable risk calculations to be made even though a surrogate itself may not necessarily be present within the study area. However, all of the receptors are present in Washington State and are representative of feeding guilds present. The fundamental assumption that was made in this assessment was that if negligible risk is determined for the surrogate species, then the entire guild is protected.

Specific receptors were selected based on the evaluation of exposure pathways and the possibility that a given receptor could come into contact with imazamox via Japanese eelgrass treatment. The receptor selections were limited mainly to those receptors found in the areas where Japanese eelgrass is distributed, and to surrogate species for which sufficient life history and/or toxicological information existed so that reasonable exposure factors could be used to estimate exposure and risk. The following bullets briefly summarize the receptors for which exposure and risk were evaluated. Life history characteristics of these receptors are described fully in **Section 4**.

- **Mallard (*Anas platyrhynchus*)**. Mallards are representative of primarily herbivorous waterfowl. The mallard was evaluated due to its direct and indirect exposure through the consumption of aquatic plants.
- **Bobwhite Quail (*Colinus virginianus*)**. While this species is not native to Washington, it is a common avian test species and serves as a good surrogate for the introduced California (valley) quail (*Lophortyx californica)*, which is relatively common in western Washington. This species is primarily grainivorous.
- **Norway Rat (*Rattus norvegicus*)**. Ubiquitous mammalian species found in lowland areas throughout Washington State and the U.S. The Norway rat is commonly used for toxicity testing. It represents omnivorous mammal species (e.g., shrews, moles, bats, and myotis species).
- **Red Fox (*Vulpes vulpes*)**. Medium-sized, primarily carnivorous, mammal of the canine family that is a resident to much of western Washington. It is a surrogate for other carnivorous species such as the wolf, coyote, and mustellids.
- **Green Algae (*Selenastrum capricornutum*)**. Common freshwater algae species typically used in ecotoxicology studies. *S. capricornutum* can be used as a surrogate for other aquatic algae.
- **Native eelgrass (*Zostera marina*)**. The potential effects to this vascular plant are central to the risk assessment and to the efficacy studies that have been conducted in consideration of the proposed imazamox treatment for Japanese eelgrass.
- **Marsh Wren (*Cistothorus palustris*)**. Common native avian species to coastal grasslands and salt marsh habitats in Washington State. This species consumes a high proportion of its diet in animal protein.
- **Dungeness crab (*Cancer magister*)**. Common macroinvertebrate species within eelgrass habitat, low intertidal and subtidal zones. Dungeness crab is known to control eelgrass growth through bioturbation and grazing. If specific toxicology information does not exist for *C. magister*, then mysid shrimp (*Americamysis bahia*) will be used.
- **Rainbow Trout (*Oncorhynchus mykiss*)**. Common salmonid native to Pacific Ocean tributaries. Juvenile and adult salmonids commonly use aquatic vegetation for foraging and refuge habitat. Because there is an anadromous form (steelhead), rainbow trout can be used as a surrogate for Chinook salmon and green sturgeon (*Acipenser medirostris)*.

- **Cottontail Rabbit (*Sylvilagus* spp.)**. Strictly herbivorous species common western Washington, but introduced originally from the east coast of the U.S. It is also a typical EPA test species used particularly to evaluate dermal sensitivity.

2.4.1.1 Threatened and Endangered Species and Species of Concern

Threatened and endangered (T&E) species and species of concern are those species that have been given special legal and/or protective designations by federal or state government resource agencies. A federally endangered species is one that is in danger of extinction throughout all or a significant portion of its range. A federally threatened species is one likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range. A species of concern is one for which status information suggests the species is not abundant, and for which additional information is sought.

Addressing exposure and risk to T&E species generally requires the use of surrogate receptor guilds because they are rarely used to establish toxicity information on new chemicals (Sappington et al. 2001). In brief, use of Willapa Bay by T&E aquatic species is primarily limited to the listed salmonid species from the Columbia River that may dip in to the bay, and the green sturgeon—which is known to regularly use the bay as sub-adults. Pacific eulachon (*Thaleichthys pacificus)* are not known to regularly use Willapa Bay and the bay is not designated as critical habitat for the species. In addition, several coastal avian species listed as sensitive, candidate, or state-monitor species are common to Willapa Bay and other areas where Japanese eelgrass is distributed (**Table 2-5**).

Table 2-5 List of Threatened or Endangered Species that could potentially be exposed during imazamox treatment of *Zostera japonica*.

General Taxon		Species	Status		County	
			State	Federal	Pacific	Grays Harbor
Vertebrates	Fish	Green Sturgeon (*Acipenser medirostris*)	N	T	X	X
		Eulachon (*Thaleichthys pacificus*)	C	T	X	X
		Bull Trout (*Salvelinus confluentus*)	C	T	X	X
		Chinook Salmon (*Oncorhynchus tshawytscha*)	C	T	X	X
		Chum Salmon (*Oncorhynchus keta*)	C	T	X	X
		Coho Salmon (*Oncorhynchus kisutch*)	C	T	X	X
		Steelhead Trout (*Oncorhynchus mykiss*)	C	T/E	X	X
Mammalian and Avifauna	Marine Birds	Brown Pelican (*Pelecanus occidentalis*)	E	D	X	X
		Marbled Murrelet (*Brachyramphus marmoratus*)	T	T	X	X
		Short-tailed Albatross (*Phoebastria albatrus*)	C	E	X	X
	Shorebirds	Snowy Plover (*Charadrius alexandrines*)	E	T	X	X
	Marine Mammals	Killer Whale (*Orcinus orca*)	E	E	X	X
		Steller (Northern) Sea Lion (*Eumetopias jubatus*)	T	T	X	X

D = Delisted due to Recovery, T = Threatened, E = Endangered, C = Candidate, X = Presence, N = Not designated
Source: WDFW 2008

Species that are listed within Pacific or Grays Harbor County as threatened (T) or endangered (E), but are unlikely to occur within estuary habitat include (State (S), Federal (F) listing):

Butterflies:

- Oregon Silverspot (*Speyeria zerene hippolyta*), SE, FT

Reptiles:

- Pacific Pond Turtle (*Actinemys marmorata*), SE, FS Candidate(C)

Fish:

- Bocaccio Rockfish (*Sebastes paucispinis*), listing is for Puget Sound/Georgia Basin, SC, FE
- Canary Rockfish (*S. pinniger*), listing is for Puget Sound/Georgia Basin, SC, FT
- Yelloweye Rockfish (*S. ruberrimus*), listing is for Puget Sound/Georgia Basin, SC, FT

Birds:

- Spotted Owl (*Strix occidentalis*), SE, FT
- Streaked Horn Lark (*Eremophila alpestris strigata*), SE, FC
- Western Gray Squirrel (*Sciurus griseus*), ST, FSC
- Western Pocket Gopher (*Thomomys mazama*), ST, FC

Mammals:

- Fisher (*Martes pennant*), SE, FC
- Blue Whale (*Balaenoptera musculus*), SE, FE
- Sperm Whale (*Physeter macrocephalus*), SE, FE

2.5 ANALYSIS PLAN FOR CHARACTERIZING RISKS TO ECOLOGICAL HEALTH

This section outlines the specific methods employed to assess risk to the ecological receptor populations identified in the conceptual model that have higher potential for exposure to imazamox in the Clearcast formulation.

2.5.1 Ecological Toxicity Risk Assessment Methods

The approach used in this SLERA follows federal guidance for conducting ecological risk assessments (EPA 1998). Briefly, the approach involves:

- Identification of chemicals of potential concern (COPCs)
- Selection of toxicity reference values (TRVs) for the COPCs
- Identification of habitats, biological communities, and biological receptors of potential concern where exposure to COPCs could occur
- Identification of exposure parameters and appropriate uptake equations
- Prediction of estimated exposure to COPCs
- Comparison of estimated exposure to recognized toxicological hazards associated with the COPCs to characterize risks

The COPCs and TRVs are identified and summarized in **Section 3** (Effects Assessment), following the literature review on the substances that could be released from the different proposed treatments. The COPCs include the active ingredients of the herbicide formulations, as well as some of the inert ingredients that may be used in the treatments.

Exposure is calculated from the general equation below (**Equation 2-1**):

Equation 2-1	**Daily intake = CM * CR * FI * AF/BW**

Where

BW = Body Weight

CM = Concentration of contaminant in exposure media(s) of concern.

CR = Contact Rate—The estimate of the quantity of the medium consumed (or otherwise taken in) per day

FI = Fractional Intake—The fraction of time (site use factor) spent in contact with the contaminated media (e.g., the proportion of the total diet obtained from the site, as extrapolated from information such as home range data on the species, or empirical findings)

AF = Absorption Fraction—The amount of contaminant contacted (e.g., consumed) that is actually assimilated into tissue to assert a potentially toxic effect

Recognizing that the contact rate may represent the additive uptake by several pathways (e.g., ingestion of treated animal, plant, and soil or sediment matter) requires the estimate of the additional dose from other exposure media. These modifications, along with the input parameters necessary to gauge dose to the array of ecological receptors modeled, are detailed in **Section 4** (Exposure Assessment).

To characterize risk from the estimated exposure, a hazard quotient is calculated by dividing the dose received, by the chronic or acute TRV—whichever was available from the literature. For obligate aquatic species, risks were characterized by using the estimated concentration of imazamox formulation constituent with complete mixing as the EEC and dividing that by the TRV identified in the literature, as identified in **Equation 2-2**.

Equation 2-2	**HQ = EEC/TRV**

Where

EEC = Environmental Exposure Concentration or Dose (i.e., the concentration of contaminant in the exposure media)

TRV = Toxicity Reference Value by exposure pathway,

The calculation of HQs, by species, represents the culmination of the exposure and effects assessments, and these metrics are provided in **Section 5** of this report (Risk Characterization). Where data by multiple exposure pathways are available, hazard quotients are summed across exposure pathways to calculate hazard indices (HI) to represent cumulative exposures to each receptor.

3 Toxicity Hazard Assessment

The toxicity hazard assessment portion of this SLERA summarizes environmental fate, environmental persistence, and toxicity data developed for imazamox. Additionally, toxicological literature on imazamox as proposed to be used to control Japanese eelgrass is reviewed in this section to determine the most appropriate TRVs. By comparing estimated exposure doses to TRVs, it is possible to estimate the potential risks to ecological receptors from treatment chemicals identified for each alternative.

A central tenet of toxicology of non-carcinogenic compounds is that at some exposure dose no effect is measurable in the response tested, and this paradigm is considered a valid model for this assessment. This dose or concentration is known as the no observable adverse effect level or concentration (NOAEL or NOAEC). The lowest observable adverse effect level or concentration (LOAEL or LOAEC) corresponds to the lowest dose at which a statistically significant difference is measurable relative to an unexposed control group. Beyond these typical measures, standard toxicological terms include the LC_{50}, the exposure concentration that kills 50% of the animals tested and the EC_{50}, the concentration that elicits a non-lethal effect in 50% of the organisms tested with the measurement endpoint. Measures such as the LC_{90} or EC_{90} simply reference variations in the proportion of the population tested that responds to the test (in this case, 90%). Other terms such as the IC_{50} or IC_{10}, reference a concentration that results in inhibition of an endpoint—in this case 50% and 10% inhibition respectively. These terms are often used to gauge the effect of a chemical on endpoints such as growth, or in-vitro endpoints such as the inhibition of an enzyme.

Hazard statements are used in product labeling as a means of risk communication to the public. For example, **Table 3-1** summarizes chemical hazard classifications based on mammalian toxicity testing results for the protection of *human health* as specified in EPA's Office of Pesticide Programs. (Hazard criteria specific to fish and wildlife are discussed later in **Section 3.3**).

Table 3-1 Hazard Statements for Human Health Hazard Classification, based on Surrogate Mammalian Toxicity Tests

Hazard Category	Mammals		
	Acute Oral LD_{50} (mg/kg)	Acute Dermal LD_{50} (mg/kg)	Acute Inhalation (mg/L)
Category I: DANGER	< 50 Fatal if swallowed	< 200 Fatal in contact with skin	< 0.5 Fatal if inhaled
Category II: WARNING	>50 < 500 May be fatal if swallowed	>200 < 2000 May be fatal in contact with skin	>0.05 < 0.5 May be fatal if inhaled
Category III: CAUTION	>500 < 5000 Harmful if swallowed	>2000 < 5000 Harmful in contact with skin	>0.5 < 2.0 Harmful in inhaled
Category IV: CAUTION	> 5000 No hazard statement required	> 5000 No hazard statement required	>2 No label elements required

The fate and transport information summarized in this section allows for an evaluation of the expected mobility, degradation and persistence of the chemicals associated with the proposed treatment and alternatives, in both abiotic media (e.g., soil, water, air) and biotic media (biological tissues). Persistence of chemicals in biological tissues is commonly characterized through bioconcentration or bioaccumulation. Bioconcentration of a chemical can occur in an organism when it accumulates chemicals in its tissues following direct exposure, at a concentration greater than that found in the exposure media (e.g., water, air, soil, sediment). If the organism is then consumed (i.e., predated upon) by another organism resulting in a higher concentration of the chemical in the predator, then the chemical is considered to bioaccumulate. Thus, these terms

should not be interpreted to be synonymous as they reference different properties that can be of significance when characterizing chemical hazards.

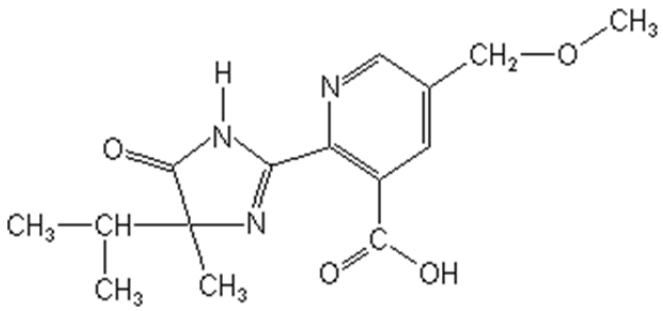
Ney (1998) explains that bioaccumulation of organic chemicals in animals is a function of a chemical's ability to become soluble with fat. Fat-soluble (hydrophobic, nonpolar) chemicals are more prone to bioaccumulate in fatty tissues of animals because they are less prone to be metabolized by animals and will not, or will only slowly, dissipate or depurate when the animal is no longer exposed to the chemical. Chemicals that are insoluble in lipid exhibit polarity, and are readily metabolized, dissipated, and depurated. The bioconcentration factor (BCF) is principally a measure of the tendency for a substance in water to accumulate in organisms, especially fish. It is the ratio of the concentration of a chemical inside an organism to the concentration in the surrounding environment (Ivanciuc et al. 2006).

Inert ingredients are mixed with herbicides that increase the binding and/or uptake into the target species. Typical inert ingredients include surfactants, wetting agents, spreaders, emulsifiers, dispersing agents, solvents, and solubilizers. Inert ingredients 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. Although little difference appears to exist among inert ingredients in their potential efficacy, their inherent chemical properties can have a range of environmental issues that are independent of the herbicide formulation in which they may be applied. Inert ingredients are components within the patented product formulations that are reported to have no herbicidal activity.

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) regulations do not require manufacturers to reveal the inert, adjuvant, or surfactant ingredients contained within commercial product formulations registered for use by the EPA. FIFRA regulates the active ingredients only, through toxicity testing of the product formulations in their entirety. The potential additive or synergistic effects of any additional constituents in formulations are therefore considered implicit to the toxicity testing results. Furthermore, the inert and surfactant ingredients in commercial formulations of the various herbicide products on the market are often not known and are protected by law from disclosure. Notwithstanding, it is our understanding that no surfactants or adjuvants are included in the Clearcast formulation considered under the proposed program, though the product label reports inert ingredients.

3.1 CHEMICAL FORMULATION

Imazamox ($C_{15}H_{19}N_3O_4$) (CAS number 114311-32-9) is a broad spectrum imidazolinone herbicide, which is in the same family of herbicides as imazapyr (e.g., Arsenal®), imazapic (e.g., Cadre®), imazathapyr (e.g., Pursuit®), and others (Grey 2009). The structure of imazamox is provided on **Figure 3-1**. Imazamox was first registered under the commercial formulation Raptor® in 1997 by the American Cyanamid Company (now part of BASF Corporation) (EPA 2009a). It was developed for use on alfalfa, edible legumes, and soybeans in agricultural and domestic applications to control early post-emergence of annual grass and broadleaf weeds via ground or aerial application in an aqueous solution (EPA 1997). In addition to use in agricultural systems, imazamox was also under an experimental use permit (EUP) from the EPA starting in 2005 for evaluation in aquatic systems (Wersal and Madsen 2007). As a result of experimentation completed with the imazamox product Clearcast, the EPA released a full Section 3 Aquatic Use Label on March 20, 2008 for use on submerged, emergent and floating vegetation (EPA 2009a).



2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid

Figure 3-1 Chemical Structure of Imazamox
Source: EC 2002

Imazamox has been produced under different commercial formulations, as diluted forms of ammonium salt of imazamox, resulting in a weak acid solution (**Table 3-2**). The formulations have the same mechanism of action on target plants, but different environmental factors control the efficacy of each formulation and where they might be applied. Most testing related to the toxicity of imazamox is related to the technical compound instead of the commercial formulation. Clearcast listed below is the formulation projected for use in the estuary setting for Japanese eelgrass control, which is currently approved for use in freshwater and marine systems. Mechanism of action, environmental fate, and toxicity studies described in detail below reference these general formulations.

Table 3-2 Product Formulations of Imazamox Currently Registered with the EPA

Commercial Product	% Ammonium Salt	% Inert Ingredient	Controls Vegetation In and Around	EPA Registration #	Source
Raptor® Herbicide Technical	97.4	2.6	NA	241-378	EPA 2009a
Raptor® WG Herbicide	70.0	30.0	field peas, legume-based pastures, lucerne, peanuts, soybeans	241-380	EPA 2009a, CropCare 2006
Raptor® Herbicide	12.1	87.9	alfalfa, chicory, clover seed, lima beans, peas, snap beans, soybeans	241-379	EPA 2009a, BASF 2008a
Beyond® Herbicide	12.1	87.9	Clearfield canola, sunflower, wheat	241-379	EPA 2009a, BASF 2006
Clearcast® Herbicide	12.1	87.9	aquatic and noncropland sites	241-437	EPA 2009a, BASF 2008b

3.2 ENVIRONMENTAL CHEMISTRY AND FATE

3.2.1 Chemical and Physical Properties

Physical and chemical properties of imazamox are presented in **Table 3-3**. Imazamox is ionized under typical environmental conditions of pH 5 to 9, and is therefore highly soluble in water. Additionally, the solubility of imazamox increases with water temperature. For example, the solubility of the compound is reported as 4.41 g/L and 116 g/L at 20°C and 25°C, respectively (EPA 1997, EC 2002). Typical temperatures of application in Washington State would bracket solubility measures recorded between about 15°C and 25°C.

Table 3-3 Physicochemical Properties of Imazamox (Parent Compound)

Parameter	Value(s) and Conditions	Source
Odor	Odorless	EPA 1997
Molecular weight	305.33 g/mol	EC 2002
Melting point	Technical 97.1: 166.0-166.7°C Purified 99.5: 165.5-167.2°C	EC 2002
Relative density	1.39 (99.3%)	EC 2002
Vapor pressure	<1.33x10-5 Pa at 25°C	EC 2002
Henry's law constant	<9.76x10-7 Pa m3 mol^{-1} at 25°C	EC 2002
Solubility in water	4413 mg/L at 20°C pH 5: 116 g/L at 25°C pH 7: >626 g/L at 25°C pH 9: >628 g/L at 25°C	EPA 1997, EC 2002
Solubility in organic solvents	Hexane: 0.007 g/L Methanol: 67 g/L Toluene: 2.2 g/L Ethyl acetate: 10 g/L	EC 2002
Octanol-water partition coefficient (log K_{ow})	pH 5 & 6: 5.36 at 25°C pH 5 & 6: 0.73 (corrected for dissociation)	EPA 1997 Agropages 2009
Dissociation constant (pK_a)	pH 5: 3.3 pH 7: 2.3 based on technical grade, not pure active constituent	NRA 2000
Hydrolytic stability (DT50)	DT50 = 192 days at 25°C, pH 9 no hydrolysis at pH 4 and 7	EPA 1997, EC 2002
Quantum yield of direct photo-transformation in water at ε >290 nm	6.13x10-3 mol einstein-1 at 20°C	EPA 1997, EC 2002
Photostability in water (DT50)	pH 5: DT50 = 6.8 hr pH 7: DT50 = 6.7 hr pH 9: DT50 = 7.1 hr	EPA 1997, EC 2002

The dissociation constant (pK_a) reported for imazamox (the pH at which an acid is 50% dissociated between its anionic and ionic form) reflects its ionization potential under typical environmental conditions. When the pH of a solution is equal to its pK_a, the chemical will be dispersed equally between an anionic and ionic state. In general, ionized forms of chemicals represent lower ecological risk because they are unable to penetrate cell membranes due to low lipid solubility. For weak acids, such as imazamox, as the pH is elevated above the pK_a, the proportion of the compound in an ionic state will increase.

3.2.2 Environmental Fate and Persistence

The rate and form of degradation of imazamox varies somewhat with the environment where it is applied. Movement within the environment (e.g., through soils, water, plants) of a weak acid is primarily determined by the pH of the host system. For example, the primary form of degradation in water is via photodegradation. Photolysis half-lives in water have been reported at 6.8 hours (EPA 1997); however, degradation decreases with increasing pH. Further, pH controls the amount of accumulation of a weak acid in plants, whereby lower pH increases an herbicide's relative toxicity due to lower ionization of the herbicide and increased uptake by the plant (Trapp 2000). In soils, degradation is primarily driven by microbial metabolism, which also appears to decrease with increasing pH and low soil moisture (Ball et al. 2003). Microbial metabolism in sediments has not been thoroughly investigated, although some basic trends are provided below.

3.2.2.1 Environmental Fate and Persistence in Vegetation

Absorption of imazamox in plants occurs through foliage and roots (EPA 1997, Trapp 2000). In an acidic environment (low pH), weak-acid herbicides are in an anionic state and can freely cross plant membranes (**Figure 3-2**) (Petroff 2005). Once the herbicide is absorbed through the roots, the phloem is an alkaline environment (high pH) and the herbicide becomes ionized, which effectively traps the compound in the phloem and thus the root system (Trapp 2000). Any anionic form of the herbicide can move freely between the xylem and phloem, and because the xylem moves faster, net movement is toward the leaves (a.k.a., translocation). Reduced uptake of the herbicide by target plants is due to the alkaline nature of the plant surface and the level of dissociation of weak-acid herbicides (Petroff 2005). Broad-leaved plants have alkaline leaf surfaces and the use of an acidifier in spray solutions can effectively neutralize higher pH values on leaf surfaces. Eelgrass, in contrast, has a thin, highly permeable cuticle and no stomata. This structural difference is presumably what allows for the proposed use of imazamox without an additional surfactant.

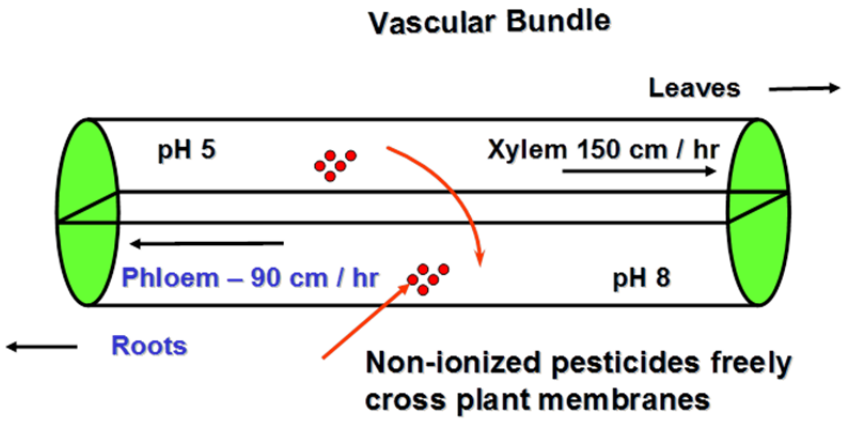


Figure 3-2 Movement of Ionizable (Weak-Acid) Herbicides into Plant Tissue
Source: Petroff 2005

Absorption and translocation of imazamox in plants varies depending on the species and environmental conditions. For example, parrotfeather (*Myriophyllum aquaticum*) does not fully translocate imazamox throughout the plant, thereby reducing the efficacy of the herbicide (Wersal and Madsen 2007, Mateos-Naranjo et al. 2009). Imazamox caused necrosis of the apical meristems; however, emergent shoots grew from the nodes beneath the dead shoots by 6 weeks post treatment. Pester et al. (2001) commented that the differential response of jointed goat grass and feral rye to imazamox was related to differences in translocation and metabolism rather than absorption.

A separate study in freshwater that may effectively represent estuarine conditions was completed by Cedergreen et al. (2005) in surface waters flowing at a rate of 0.5 mL/min. The authors compared area-specific growth effects of *Lemna minor* (a floating macrophyte) in response to 3-h pulse (followed by growth in herbicide-free media) and long-term (4 and 7 day) media treatments of the Bolero™ formulation of imazamox. Growth of *L. minor* decreased in a similar pattern in response to increasing chemical exposure under both the 3-hr pulse and long-term time periods, albeit at a slower pace for the pulse treatment (**Figure 3-3**). This was also observed in the associated EC_{50} values for each time period. For example, the EC_{50} for a 4-day experiment was 1080±110 nM for the 3-hr pulse exposure and 179±22 nM for the 4-day media exposure. However, exposure time did not make a difference at the highest concentration tested (10 µM) for the 4-day experiment, which resulted in the same arrestment of growth/mortality for both the pulse and media

exposure. Overall, the authors concluded that compounds with a high octanol-water partition coefficient (K_{ow}) enter plants rapidly and have a greater effect when applied in pulses.

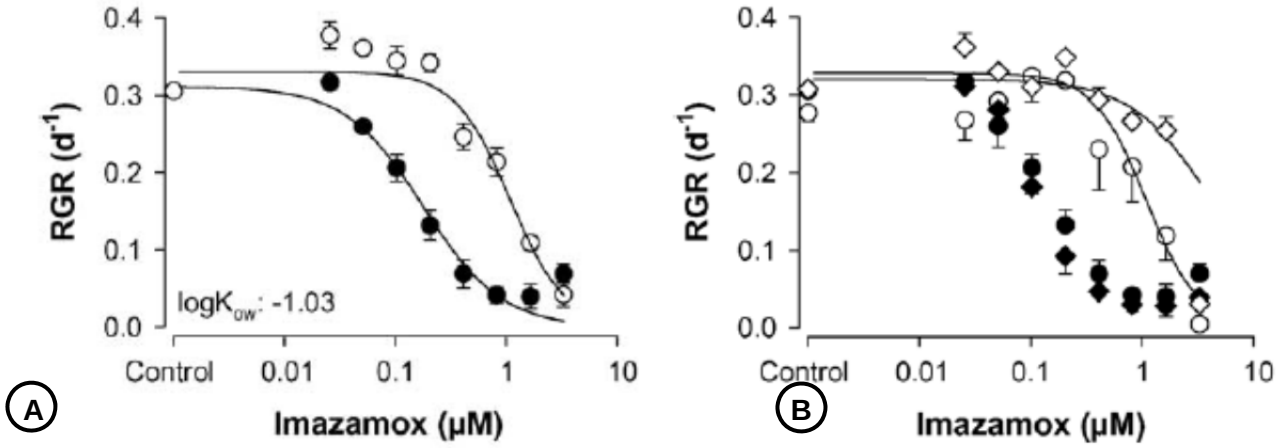


Figure 3-3 Area-Specific Relative Growth Rates (RGR) of *Lemna minor* as a Function of Imazamox Herbicide Concentration: After 4 Days (A) and After 4 and 7 Days (B)

Note: The plants were either kept in media with herbicide (filled symbols) for 4 or 7 days or were exposed to the herbicide for 3 h (open symbols) after which they were left to grow in herbicide-free media. Circles (○ or ●) represent measurements after 4 days. Diamonds (◊ or ♦) represent measurements after 7 days. Source: Cedergreen et al. 2005.

According to a 3 year study of Japanese eelgrass by Patten (2008), management of eelgrass starts to break off at 16-20 oz/ac and 32 oz/ac provided 100% efficacy in each preliminary trial (Appendix A). However, when conditions included pooled or flowing water, no effective management was observed. The same observation was made for submerged application of imazamox to native eelgrass. In addition, control was most effective from April to September (Patten 2008), which coincides with vegetative growth and flowering of Japanese eelgrass shoots (Harrison 1982b).

3.2.2.2 Environmental Fate and Persistence in Soils

Imazamox is moderately persistent in soil, and degrades aerobically to a non-herbicidal metabolite (EPA 1997, Aichele and Penner 2005). According to various studies on adsorption of imazamox in soil, it will weakly attach to sediment particles in its initial form (EPA 1997, Celis et al. 1999, EC 2002, Ball et al. 2003, Aichele and Penner 2005). Thus, the parent compound is mobile in soil. However, the terminal metabolite is moderately mobile to immobile (EPA 1997), which indicates that it has limited leaching potential once degradation occurs. Adsorption is both pH- and water-dependent, in that the bioavailability of imazamox increases at low pH and at low levels of soil moisture (Ball et al. 2003).

The primary degradation pathway for imazamox in soils is through microbial metabolism, with photolysis and other degradation pathways providing a limited source of degradation (Aichele and Penner 2005). During aerobic microbial metabolism, the imidazolinone ring is opened and a hydroxy metabolite is formed as a result of the conversion of the carboxilic acid group on the pyridine ring (Mangels and Ritter 2000). According to a study that measured the soil persistence of imazamox on dryland crops in the Pacific Northwest (Ball et al. 2003), insufficient soil moisture was found to limit decomposition of imazamox, presumably by limiting microbial degradation.

A review of degradation studies worldwide by the European Commission (EC 2002) indicated that the half-life (or stability) of imazamox in soil ranges from 12 to 207 days at a pH ranging from 5.8 to 8.1 (**Table 3-4**). The reported range of persistence in Pacific Northwest soils is 90 to 780 days, though no pH range was provided (Thill et al. 2008). The lowest half-life reported was from Aichele

and Penner (2005) under laboratory conditions (pH 7, capac sandy loam soil, 0.21-0.27 g water/g soil, application rate of 0.23 µg/g, 22ºC), which resulted in a half-life of 1.4 wk for imazamox. It is notable that, given the same physical conditions, degradation slows down as the compound reaches the final metabolite (**Figure 3-4**). Microbial degradation of imazamox in soils appears to decrease with decreasing pH; soil pH below 6.2 has been found to reduce the sorption onto sediment particles and increase the persistence of imazamox (Celis et al. 1999, Ball et al. 2003, Aichele and Penner 2005). As a result, agricultural plots treated the previous year with imazamox can cause injury to newly planted crops.

Table 3-4 Rate of Degradation of Imazamox in Different Soil Types

Soil Type	OC (%)	pH (w)	Degradation (d)
DT50lab (20°C, aerobic)			
Sandy loam	1.5	6.8	45*
Sandy loam	1.7	-	40*
Silt loam	0.8	5.8	207
Silt loam	0.8	6.5	44
Silty clay loam	1.1	8.1	12
DT90lab (20°C, aerobic)			
Sandy loam	1.5	6.8	149
Sandy loam	1.7	-	133
Silt loam	0.8	5.8	687
Silt loam	0.8	6.5	147
Silty clay loam	1.1	8.1	39
DT50lab (10°C, aerobic)			
Silt loam	0.8	6.5	113
Silty clay loam	1.1	8.1	42
DT50lab (20°C, anaerobic)			
All compounds are stable, degradation in the saturated zone			

Source: EC 2002

*adjusted to 20°C (Arrhenius, Ea 54 kJ mole-1) slower degradation at acidic pH

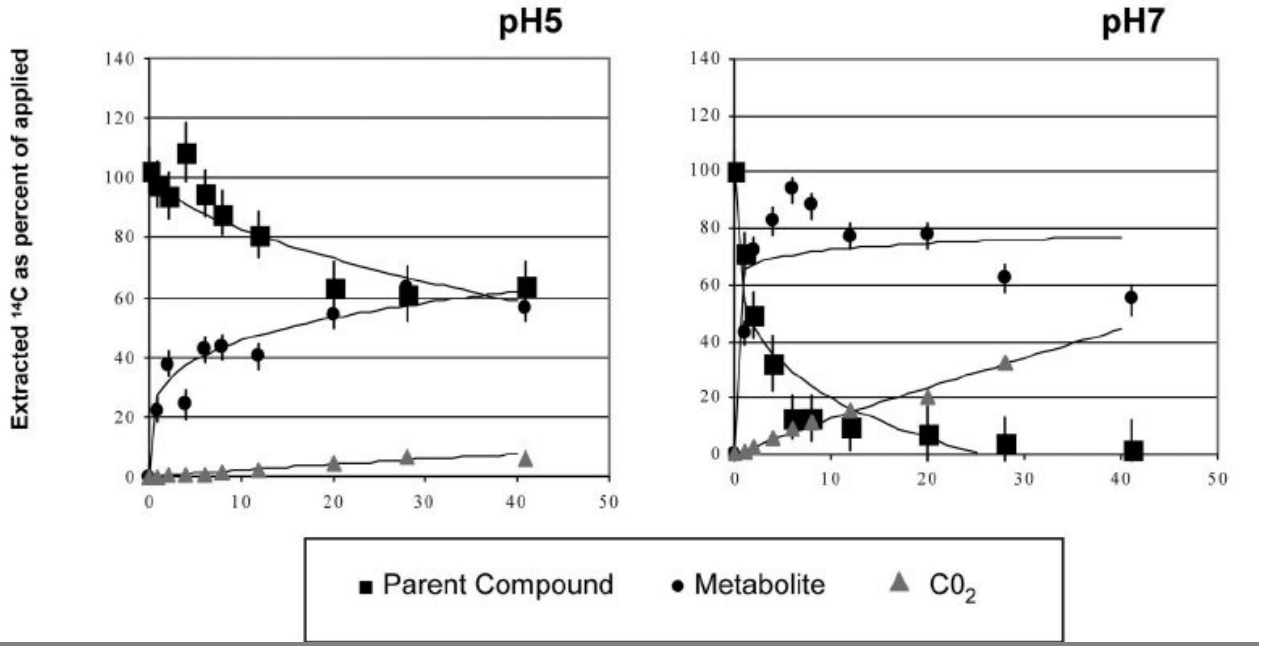


Figure 3-4 Results of the degradation of imazamox in soil at a pH of 5 and 7. Each line represents the disappearance of the parent compound or the appearance of metabolites.

Source: Aichele and Penner 2005

Manipulating the adsorptive capacity of different soil types for herbicides has been modeled or tested by a number of researchers. For example, Cruz-Guzman et al. (2005) tested the ability of organic cations to influence the adsorptive capacity of clay minerals for organic compounds, such as pesticides and herbicides. Although imazamox was not tested, a related compound (imazathepyr) was shown to have an affinity for cystine-treated bentonites. One of the key elements in the experiment was that pH values of the sediment suspensions were higher than the pK_a value of imazethapyr, which resulted in an anionic form of the compound. That, combined with cystine cations created an ionic bond between the sediment and herbicide. Therefore, the addition of certain organic cations can optimize adsorption of organic pollutants and immobilize or delay their movement toward groundwater.

In a study testing the adsorptive properties of imazamox specifically, Celis et al. (1999) reported that the ionic properties of the sediment particles and the pH of the environment have significant implications for the amount of sorption and the strength of the bonds. For example, at the typical environmental pH of 6 to 7, imazamox was in an anionic state and the calcined product of HT (HT500) was the best sorbent. In contrast, decreasing the pH (<5.5) increased sorption capacity of organoclays for imazamox, which in its ionic state had a greater affinity for the interlayer organic phase of the mineral. In addition, the Celis et al. (1999) study concurred with the previous study, in that replacement of natural metal-exchange cations with organic cations changed the nature of clay surfaces from hydrophilic (negatively charged) to hydrophobic (positively charged), which then made it possible to adsorb imazamox in its anionic state. This is due to the charge of sediment particles compared to the charge of imazamox in the environment (**Figure 3-5**), where two molecules that are oppositely-charged (i.e., cation and anion) will form an ionic bond, but two similarly charged particles will repel each other.

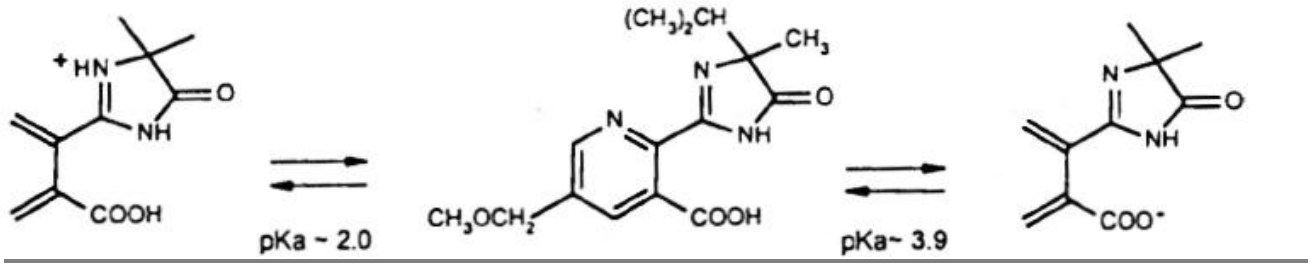


Figure 3-5 Imazamox Acid-Base Equilibrium
Note: pKa values are for imazethapyr not imazamox. Source: Celis et al. 1999

3.2.2.3 Environmental Fate and Persistence in Water

When imazamox is applied directly to water, its degradation largely mimics the pathway by which the herbicide would be degraded at high tide after application to Japanese eelgrass during low tide. Residual imazamox on plants that may not be completely absorbed will be inundated by the incoming tide within 1 to 3 hours after application (depending on the time of application, and the tidal stage on the day and time of application), solubilized, dispersed and further degraded. In general, the parent compound of imazamox degrades relatively quickly in water; however, the derivatives (metabolites) may remain in solution for 50-100 hrs (Mateos-Naranjo et al. 2009).

Photolysis is a principal means of imazamox degradation in water (Quivet et al. 2006, Harir et al. 2007). Harir et al. (2007) reported a half-life of imazamox in water of 8.3 hrs, compared to 1.3 hrs from Quivet et al. (2006). These differences are most likely due to the pH of the photodegradation solution, which was 5.0 and 2.6 in the Harir et al. (2007) and Quivet et al. (2006) studies,

respectively. Harir et al. (2007) reported that imazamox degrades rapidly in freshwater by photolysis into five photoproducts (and 9 minor photoproducts). In contrast, Quivet et al. (2006) reported six photoproducts and no minor products.

Both studies reflect that photodegradation in water begins with the opening of the imidazolinone ring and the loss of either the lactam C═O (decarbonlylation process; **Figure 3-6**), or the loss of a H_3CNO fragment from the parent compound (transposition reaction; **Figure 3-7**). However, Harir et al. (2007) reported two potential main pathways of degradation that corresponded to two primary metabolites, while Quivet et al. (2006) reported that loss of the lactam C═O is always the first step and further degradation from that product occurs through either the loss of an amino group or the hydration of the alkene group.

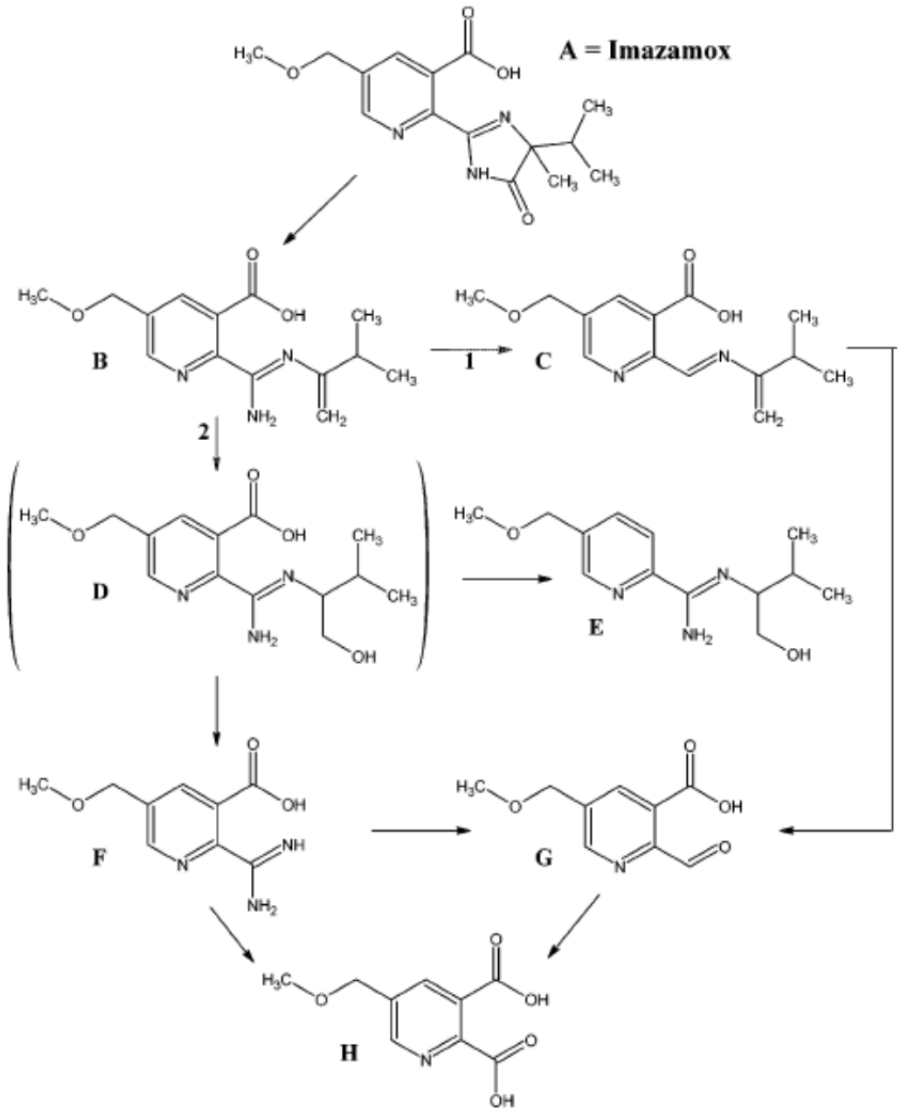


Figure 3-6 Proposed Pathway for Imazamox Photodegradation in Water
Note: Based on mass spectroscopy analysis. Source: Quivet et al. 2006

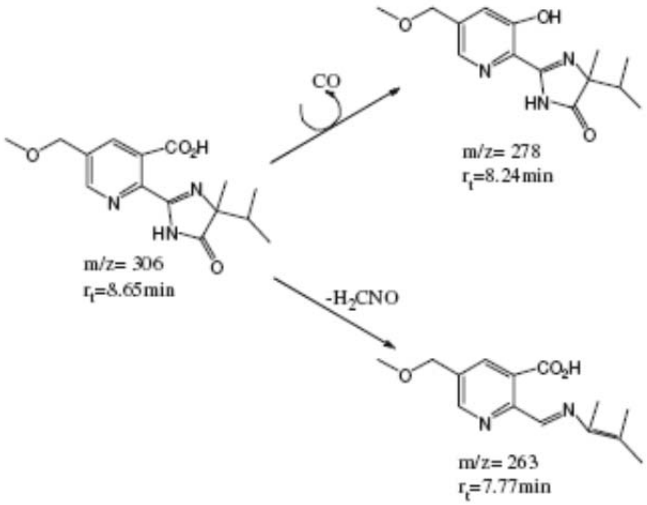


Figure 3-7 **Proposed Structural Formulations of the Two Main Photoproducts from Imazamox Photolysis Note: m/z = mass to charge ratio (amu), rt = retention time. Source: Harir et al. 2007**

Quivet et al. (2006) also explored the effect of copper chloride ($CuCl_2$), calcium chloride ($CaCl_2$), copper nitrate ($Cu(NO_3)_2$), and calcium nitrate ($Ca(NO_3)_2$) on imazamox degradation. The presence of copper ions (i.e., Cu^{2+}) decreased the imazamox degradation rate from 78 minutes to >400 minutes (**Figure 3-8**), presumably through complexation. Calcium ions (Ca^{2+}) also decreased the degradation rate (>150 minutes), although not to the extent of copper because copper(II) complexes are generally more stable than calcium(II) complexes. In contrast, chloride ions (Cl^-) were not shown to have a significant effect on degradation, whereas nitrate ions (NO_3^-) led to photooxidation and accelerated the degradation of imazamox.

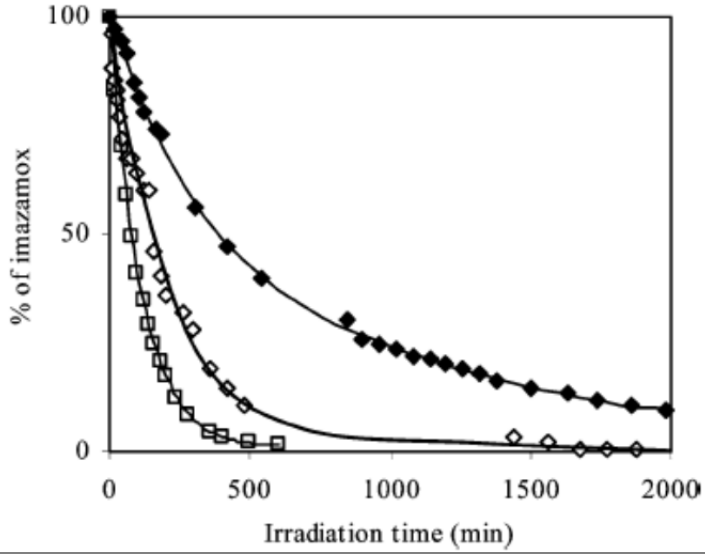


Figure 3-8 **Degradation Kinetics for 10 mg/L Imazamox without Metal Salts (□) and in the Presence of CaCl2 (◊) or CuCl2 (♦)**

Note: pesticide to metal salt molar ratio R = 1. *Source*: Quivet et al. 2006

3.2.2.4 Summary of Environmental Fate and Persistence

Table 3-5 summarizes draft criteria for environmental fate, as recently outlined by the EPA Design for the Environment Program (EPA 2011). These criteria are problematic for assigning a category to the intended use of imazamox given the intertidal conditions proposed. Based on soils criteria, and the data outlined in **Table 3-4**, the herbicide would be considered moderately to highly persistent. Based on water criteria, and data outlined in **Section 3.2.2.3**, its persistence would be considered very low. Thus, such criteria as put forward by the EPA in this case leave much room for clarification and further development.

Table 3-5 Criteria for Persistence Designations

Environmental Persistence	Very High	High	Moderate	Low	Very Low
Persistence in water, soil or sediment	Half-life > 180 days	Half life of 60 to 180 days	Half life less than 60 days but greater than or equal to 16 days	Half life less than 16 days, or passes ready biodegradability test not including 10-day window	Passes ready biodegradability test with 10-day window

Source: EPA 2011—Alternatives assessment criteria for hazard evaluation, EPA Design for the Environment Program, Version 2.0.

In the marine intertidal mudflats where imazamox would be applied to control Japanese eelgrass, the pH of sediment surfaces and sediment pore water range from 6.8 to 7.6 in Grays Harbor and 7.3 to 7.6 in Willapa Bay (Wilson and Partridge 2007). Imazamox dissociates at pH of 5 and 7 (equivalent to a pK_a of 2.3 and 3.3); thus, it would be in an ionic state in these estuaries, highly soluble and highly unlikely to persist. Site specific studies were conducted to clarify its environmental persistence and to identify EECs in water and sediments. The resulting data suggest that imazamox would have very low to low persistence in the Willapa Bay environment where the proposed applications would occur.

3.2.3 Toxicity to Wildlife

Toxicity to wildlife is defined by what the chemical is engineered to do and if the same pathway exists from exposure to non-target wildlife species (mechanisms of action), the rate at which a chemical may accumulate in tissue (bioconcentration and bioaccumulation), and how much is potentially absorbed and processed (metabolism). The following text describes these toxicity terms in more detail in relation to how imazamox interacts with wildlife.

3.2.3.1 Mechanism of Action

The mechanism of action of an herbicide is defined as the biochemical and/or physical method by which it has been engineered to kill or suppress the growth of specific plants. The specificity of an herbicide for target vegetation varies by herbicide family. Imazamox belongs to the chemical family imidazolinone. The imidazolinones are non-selective herbicides used to control weeds, broadleaved herbs, and woody species. Imazamox is primarily adsorbed through plant tissue, but can also be adsorbed through roots in the soil. The compound is translocated in the xylem and phloem to the meristematic tissues (WSSA 2002 *as cited in* Wersal and Madsen 2007). The mechanism of action is through inhibition of branched-chain amino acid synthesis. Specifically, imidazolinone herbicides inhibit acetohydroxyacid synthase (AHAS) or acetolactate synthase (ALS) enzymes, which then results in a lethal decrease in protein synthesis and causes foliar chlorosis and necrosis (Cox 1996, Haukkapää et al. 2005, Wersal and Madsen 2007).

Under aquatic conditions, reports vary on the efficacy for aquatic plants. For example, imazamox was found to be less effective at both initial control and maintaining control of *M. aquaticum* than imazapyr (Wersal and Madsen 2007). The authors attributed the difference to metabolism of the

herbicides into non-toxic metabolites. In other words, imazamox was not fully translocated throughout the plant before the active ingredient was broken down, and so regrowth was evident. In contrast, Patten (2008) reported higher efficacy of imazamox on Japanese eelgrass compared to imazapyr. However, conditions such as application timing, site drainage, and tidal energy influenced the long-term control and selectivity of target species.

Because aquatic plants have been shown to go into a long period (e.g., weeks) of arrested growth (Shaner 1991 as cited in Wersal and Madsen 2007), complete control may be possible with reapplication. In support of this hypothesis, Cedergreen et al. (2005) showed that compounds with a high K_{ow} enter plants rapidly and have a more detrimental effect when applied in 3-hr pulses.

Animals do not synthesize their own three branched-chain aliphatic amino acids, but obtain them by eating plants and other animals; therefore the engineered mechanism for plant toxicity is not generally relevant to birds, mammals, fish or invertebrates. Toxicity associated with excessive doses administered to animals occurs by different mechanisms.

3.2.3.2 Bioconcentration and Bioaccumulation

Biological tissues may act as an additional reservoir for chemicals applied intentionally or inadvertently to the environment. When an organism accumulates chemicals in its tissues following direct exposure it is known as bioconcentration. If the chemical accumulates at a rate faster than normal metabolic processes eliminate it, it is considered to bioaccumulate. If the organism is consumed (predated upon) by another organism resulting in a higher concentration of the chemical in the predator, the chemical is considered to biomagnify in the food web. Although bioconcentration and bioaccumulation may have toxicity implications, toxicity varies by chemical and dose, thus these mechanisms should be considered independently when evaluating the biological fate of applied herbicides. As indicated in the discussion of physical chemistry (**Section 3.2.1**) the low K_{ow} and its high water solubility indicate that imazamox is very unlikely to concentrate in tissue. According to AECOM (2009), the maximum BCF reported in fish was 0.14, which indicates that imazamox was marginally absorbed and rapidly excreted.

3.2.3.3 Metabolism

Imazamox is rapidly (~80%) absorbed and excreted by mammals. For example, when imazamox was fed to rats intravenously or orally, 80 to 90% was excreted through urine and 10 to 20% through feces within 24 hours essentially as the parent compound (EPA 1997, EC 2002). Additionally, there is no evidence of accumulation in the tissue during metabolism. Similarly, there is little concern for accumulation in aquatic animals. No bioactive metabolites inducing toxicity greater than the parent compound were identified in literature screening.

3.2.4 Wildlife Ecotoxicity Categories

Table 3-6 summarizes ecotoxicity categories for herbicide formulations identified by the EPA to provide a general gauge for comparing the potential toxicity of chemicals to mammals and birds by ingestion, and for contact exposure to non-target insects.

Table 3-6 Ecotoxicity Categories for Wildlife

Toxicity Category	Wild Mammals Acute Oral (Single Dose) LD_{50} (mg/kg)	Avian Acute Oral LD_{50} (mg/kg)	Avian Subacute dietary Concentration (mg/kg)	Non-target Insects: Acute Concentration (µg/bee)
Very highly toxic	<10	<10	<50	Not specified
Highly toxic	10-50	10-50	50-500	<2
Moderately toxic	51-500	51-500	501-1000	2-11
Slightly toxic	501-2,000	501-2,000	1001-5000	Not specified
Practically non-toxic	>2,000	>2,000	>5000	> 11

Source: www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm#Ecotox (site last updated May 9, 2012)

In many cases, no dose response was established such that the highest doses tested in animals yielded no effect, and the LD_{50} reported is simply estimated to be higher than the NOAEL identified from testing (**Table 3-7**). According to a review of imazamox by the National Registration Authority (NRA 2000), the only toxicity-related responses in animals orally treated up to 1772 mg/kg/day were slight reductions in body weight gain, reductions in the levels of white blood cells, and an increase in one liver enzyme concentration.

Table 3-7 Acute and Chronic Toxicity Reference Values to Terrestrial Receptors

Species	Pathway (Study Duration)	Exposure Dose	Study Reference	Toxicity Category
Invertebrates				
Honeybee (*Apis mellifera*)	Dermal (45 hr)	LD_{50} = >25 µg ai/bee NOAEL = 24 µg ai/bee	AECOM 2009 EPA 1994	Practically non-toxic
Birds				
Bobwhite quail (*Colinus virginianus*)	Oral (14 d)	LD_{50} = >1846 mg/kg bw NOAEL = 1846 mg/kg bw	EPA 2009b	Not ratable
	Diet (8 d)	LD_{50} = >5572 mg/kg NOAEL = 5572 mg/kg	EPA 2009b	Practically non-toxic
Mallard duck (*Anas platyrhnchos*)	Oral (14 d)	LD_{50} = >1950 mg/kg bw NOAEL = 1950 mg/kg bw	EPA 2009b	Not ratable
	Diet (8 d)	LD_{50} = >5572 mg/kg NOAEL = 5572 mg/kg	EPA 2009b	Practically non-toxic
Mammals				
Rat	Oral (acute) Oral (subchronic-28 d) Oral (subchronic-13 wk) Oral (chronic-2 yr) Inhalation (4 hr)	LD_{50} = >5000 mg/kg bw NOAEL = 1000 mg/kg bw/d NOAEL = 1661 mg/kg bw/d NOAEL = 1068 mg/kg bw/d LD_{50} = >6.3 mg/L	EPA 1997 EPA 1997 EPA 1997 EC 2002 EPA 1997	Practically non-toxic
Rabbit	Dermal Dermal irritation Eye irritation	LD_{50} = >4000 mg/kg non-to-slightly irritating Slightly-to-moderately irritating	EPA 1997 EPA 1997 EPA 1997	Practically non-toxic
Guinea pig	Dermal	Not a sensitizer	EPA 1997	Practically non-toxic
Dog	Oral (subchronic-90 d) Oral (chronic-1 yr)	NOAEL = 1368 mg/kg bw/d NOAEL = 1165 mg/kg bw/d	EPA 1997 EPA 1997	

Because application is administered from ground-based sprayers directly onto plant tissue, exposure through inhalation is not anticipated because it would be extremely unlikely that terrestrial animals would be found atop the treated mudflats during spraying. The disturbance created by the applicator would result in mobile terrestrial animals moving away from the source of disturbance, thus minimizing significant inhalation exposure potential.

Although imazamox has been shown to be practically non-toxic to the mallard in the testing summarized in **Table 3-7**, the waterfowl guild may be at greater risk to exposure due to their feeding habits on Willapa Bay tideflats. According to the Baldwin and Lovvorn (1994) study in Boundary Bay, British Columbia, Japanese eelgrass made up a significant portion of the diet of numerous migrating waterfowl. It was hypothesized that the preference of Japanese eelgrass over native eelgrass was either due to the longer accessibility during a daily tidal cycle (Japanese eelgrass occurs higher in the intertidal zone) or the higher energy content of Japanese eelgrass' leaves (18.145 vs. 16.817 kJ/g) and its smaller vegetative parts. In either case, this study suggests that waterfowl may be at the highest risk for ingestion exposure to imazamox-treated eelgrass. Further, it was commented by the authors that "birds are the only large herbivores in temperate seagrass systems," which indicates that waterfowl may be exposed to a significant portion of imazamox.

3.2.5 Ecotoxicity Categories for Aquatic Organisms

Aquatic ecotoxicity ratings can be used to generally categorize toxicity from herbicide exposure to fish and aquatic invertebrates (**Table 3-8**).

Table 3-8 Aquatic Animal Ecotoxicity Categories

Toxicity Category	Fish or Aquatic Invertebrates Acute Concentration LC_{50} or EC_{50} (mg/L)[1]	Fish or Aquatic Invertebrates Chronic Concentration NOEC or LOEC (mg/L)[2]
Very highly toxic	<0.1	<0.1
Highly toxic	0.1-1	0.1-1
Moderately toxic	>1-10	>1-10
Slightly (low) toxic	>10-100	>10
Practically non-toxic	>100	Not specified

Sources: (1)www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm#Ecotox (site last updated May 9, 2012). (2) EPA Design for the Environment Program, version 2.0, 2011.

3.2.5.1 Fish Toxicity Reference Values

Using EPA's ecotoxicity rating criteria, based on reported testing results summarized in **Table 3-9**, imazamox would be considered practically non-toxic to fish.

Table 3-9 Acute and Chronic Toxicity Reference Values in Fish

Species	Pathway (Study Duration)	Exposure Dose	Study Reference	Toxicity Category
Bluegill sunfish (*Lepomis macrochirus*)	Flow (96 hr)	LD_{50} = >119 ppm NOAEC = 119 ppm	Environmental Science & Technology 1994	Practically non-toxic
Sheepshead minnow (*Cyprinodon variegates*)	Flow (96 hr)	LD_{50} = >94.2 ppm NOAEC = 94.2 ppm	T.R. Wilbury Laboratories 1998	Not determinable, highest dose tested = NOAEC in slightly toxic category.
Rainbow trout (*Oncorhynchus mykiss*)	Flow (96 hr) Chronic (28 d) Chronic (96 d)	LD_{50} = >122 ppm NOAEC = 122 ppm NOAEC = 122 ppm NOAEC = 11.8 ppm	Environmental Science & Technology 1994 EC 2002 EC 2002	Practically non-toxic

Source: EPA 2009b – IPM Center, European Commission (EC) 2002

3.2.5.2 Aquatic Invertebrate Toxicity Reference Values

Using EPA's ecotoxicity rating criteria, based on reported testing results summarized in **Table 3-10**, imazamox would be considered practically non-toxic to aquatic invertebrates.

Table 3-10 Acute and Chronic Exposures to Aquatic Receptors

Species	Pathway (Study Duration)	Exposure Dose	Study Reference	Toxicity Category
Mysid shrimp (*Americamysis bahia*)	Flow (96 hr)	LD_{50} = >100 ppm NOAEC = 94.3 ppm	T.R. Wilbury Laboratories 1998	Practically non-toxic
Water flea (*Daphnia magna*)	Flow (48 hr)	EC_{50} = >122 ppm NOAEC = 122 ppm	Environmental Science & Tech 1994	Practically non-toxic

Source: EPA 2009b – IPM Center, European Commission (EC) 2002

3.2.5.3 Aquatic Vegetation Toxicity Reference Values

Efficacy of Clearcast (imazamox) on aquatic plants varies by the application rate utilized, the time of year of the application, the use rate, and other site-specific environmental factors (**Table 3-11**). For example, in a number of tests completed by K. Patten (2010, pers. comm.), in earlier tests, a higher rate of application (32 fluid oz/acre) was required for >90% control of Japanese eelgrass in the fall compared to a much smaller rate (4-8 fluid oz/acre) in the spring and summer when conditions were dry and only small plants were present (Appendix A). Native eelgrass growth was impacted at low doses. However, when submerged or in channels, no impacts to native eelgrass were observed by K. Patten. AECOM (2009) reported that some common emergents in wetland habitat (e.g., pickerelweed, arrowhead, bulrush, and cattails) resulted in sensitivity to water column application of imazamox, but were found to have much less sensitivity compared to terrestrial application.

Table 3-11 Acute and Chronic Efficacy Reported in Aquatic Vascular Plants and Algae

Species	Pathway (Study Duration)	Exposure Dose	Study Reference
Green algae (*Selenastrum capricornutum*)	120 h	EC_{50} = >0.037 ppm	EC 2002
Bluegreen algae (*Anabaena flos-aquae*)	Static (5 d)	EC_{50} = >0.038 ppm	Springborn Laboratories 1995
Duckweed (*Lemna gibba*)	Static (14 d)	EC_{50} = 0.011 ppm	Springborn Laboratories 1995
Freshwater diatom (*Navicula pelliculosa*)	Static (5 d)	EC_{50} = >0.037 ppm	Springborn Laboratories 1995
Marine diatom (*Skeletonema costatum*)	120 h	EC_{50} = >0.039 ppm	EC 2002
Ulva	Static, Contact	EC_{50} > 1.4 µg/cm2	Hunsperger 2010 (unpublished)

Recent unpublished tests conducted by University of Washington researchers demonstrated no significant toxic effect to sea lettuce and red algae (*Griffithsia pacifica)* when imazamox (Clearcast) was directly applied at the proposed rate of 16 oz/acre (i.e., 1.4 µg/cm^2) with a dry time of 12 hours (personal communication from H. Hunsperger to K. Patten, June 22, 2010). This dry time would be, at a minimum, four times the maximum 3-hr dry time that would be experienced in field applications under tidal conditions. These results were similar to those found by WSU researchers in field applications to sea lettuce and the red algae (*Polysiphonia hendryi*) at application rates 4-fold higher than proposed under the draft Ecology permit (i.e., 64 oz/acre) (Patten, personal communication to H. Hunsperger, June 22, 2010).

3.3 SUMMARY OF TRVS USED FOR ECOLOGICAL RISK ASSESSMENT

Toxicity reference values (TRVs) are used to evaluate potential hazards to the environment of species at risk. In the selection of TRVs, results from animal toxicity testing were used from the closest related species by exposure pathway and duration of exposure. Acute exposure TRVs were selected to represent the potential effect from the immediate application and subsequent exposure. TRVs were taken from data specific to the chemical in question (whenever available), and from tests that evaluated commercial imazamox formulations. Acute TRVs were selected from the lowest exposure dose tested in environmental media (e.g., food, water) that yielded no observable adverse effect (i.e., the NOAEL or NOAEC). These metrics were selected as a more appropriate metric than LC_{50} or LD_{50} values, which are typically used to compare against EECs to assess the potential for toxicity from acute, single-dose exposure. Typically, SLERAs would use the NOAEC or NOAEL levels to screen for potential toxicity from chronic exposure; thus, the use of the NOAEC/NOAEL as the TRV factors is conservative for considering risk potential from acute exposure because organisms typically tolerate much higher concentrations of exposure if the exposure duration is acute. The selected acute TRVs are presented in **Tables 3-7, 3-9, 3-10** and **3-11**. To consider the potential for chronic exposure risk, an additional safety factor of 0.1 was applied to the acute TRV value if no chronic exposure value was identified in testing.

The range of species for which toxicity testing has been conducted is limited, as previously discussed. Thus, additional safety (uncertainty) factors were applied to the TRVs. The general rules for applying additional safety factors for TRV values are listed in **Table 3-12**, and were applied as needed, and as indicated, in **Table 3-13**.

Table 3-12 Summary of Logic Followed for Missing Exposure Data

Situation	IF	THEN	Safety Factor
General	No NOAEC/NOAEL data for target species	Use the LOAEC/LOAEL*0.5 for target species	0.5
General	No LOAEC/LOAEL data for target species	Use the LC_{50}/LD_{50}*0.1 for target species	0.1
Within Feeding Guild	No LC_{50}/LD_{50} data for target species	Use the TRV*0.5 for related species within same feeding guild	0.5
Across Feeding Guild	No TRV for related species within same guild	Use the TRV*0.1 for closest-related species across feeding guild	0.1
Chronic Exposure	No chronic exposure data for target species	Use the acute exposure data*0.1 for target species	0.1

Table 3-13 Summary of Final TRV Values Used for Risk Screening

Species Tested	Surrogate for	Acute NOEC TRV*	Chronic NOEC TRV*
Norway Rat	Rodents raccoon	5,000 mg/kg-bw, no effect at highest acute dose tested	1,000 mg/kg-bw/day (NOAEC from 28-day study)
Dog	Red Fox, other carnivores	1368 mg/kg-bw	1368 mg/kg-bw/day
Mallard	All waterfowl	5,572 mg/kg-bw, 8-day diet study	1950 mg/kg-bw, 14-day oral study
Cottontail Rabbit	Herbivores	4,000mg/kg-bw (dermal exposure test, no dietary exposure identified)	40 mg/kg/day (no chronic value identified, safety factor applied)
Bobwhite Quail	Upland game birds and shorebirds	5,572 mg/kg-bw, 8-day diet study	1846 mg/kg-bw, 14 day oral study
Rainbow trout	All Salmonids and other coldwater fish	122 mg/L (acute exposure, 96 hr)	11.8 mg/L (96 day chronic exposure)

Species Tested	Surrogate for	Acute NOEC TRV*	Chronic NOEC TRV*
Mysid Shrimp	Dungeness crab and other invertebrates	94.3 mg/L, 96 hour acute	9.43 mg/L (no chronic data, safety factor of 0.1 applied to acute data)
Ulva/Non-target Algae	Direct Contact spray to non-target marine macroalgae	NOAEC = 1.4 µg/cm^2 (typical application rate of 16 oz a.i./acre, which is equivalent to theoretical concentration of 140 µg/L, if diluted into 10 cm depth of water)	
Duckweed	Vascular Plants, eelgrass	0.011 mg/L (i.e., 11 µg/L)— applied chronic value to acute exposure EC	0.011 mg/L (i.e., 11 µg/L)— effective concentration that yielded control in 14-day static (chronic) test.
Marine diatom (*Skeletonema costatum*)	Marine microalgae and phytoplankton	0.039 mg/L (39 µg/L). Highest dose tested--yielded no effect	

*unless otherwise specified

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4 Exposure Assessment

The purpose of exposure assessment is to characterize potential exposure of ecological receptors to imazamox as a result of its use to control Japanese eelgrass. Wildlife species are predominantly exposed to herbicides by consuming treated vegetation and/or water, and/or by transfer of the chemical through the food web. Dermal contact and inhalation exposures are possible, but are expected to be minor relative to ingestion. Inhalation exposure of drift is generally extremely limited because application equipment creates noise that causes animals to avoid the immediate area. Nocturnal animals such as the rat would largely avoid inhalation exposure because application would occur only during daylight hours.

For this SLERA, drinking water exposure to terrestrial animals would also be limited because treatments will occur in estuarine waters where freshwater is limited, although rain is generally abundant, and consumption of freshwater accumulated on the plants is possible. Exposure modeling was conducted in lieu of site specific work to gauge exposure based on application rates and delivery mechanisms of relevance to ecological receptors. Exposures were then compared to TRVs to estimate the likelihood of adverse effects.

Based on exposure modeling that reflects the method of application, inhalation exposure is not a significant exposure pathway. Similarly, dermal contact is expected to contribute insignificantly to exposure to ecological receptors. Though some marginal contribution to exposure may be possible from these pathways, accurately identifying their contribution is not possible with existing information. These limited exposure pathways are instead factored into the overall exposures through the use of safety factors.

4.1 ENVIRONMENTAL EXPOSURE CONCENTRATIONS

This section summarizes the measured EECs and, where possible, compares them to the theoretical maximum concentrations based on the proposed application rate that will likely be authorized under Ecology's NPDES permit.

4.1.1 Application Rate

For Japanese eelgrass control, only the Clearcast imazamox formulation is projected for use. Based on the draft NPDES permit language previously discussed, the application rate permitted will likely be 16 oz/acre, yielding 0.14 kg active ingredient per hectare. The herbicide will likely be applied by certified applicators.

Based on this proposed application rate, and conservatively assuming full dilution with no foliar interception or uptake, no degradation from photolysis or other means, and no sediment adsorption or penetration, a theoretical maximum concentration of imazamox of 140 µg/L is predicted in a tidally submerged depth of 10 cm on the first flood tide. A variety of empirical tests were recently conducted to provide an estimate of actual EECs. These studies, and resultant data, are summarized below to estimate the EECs anticipated for imazamox from the proposed treatments that will likely be permitted under the state-led NPDES program.

4.1.2 Water Concentrations

Actual imazamox concentrations in water will be influenced by Japanese eelgrass turion interception, adsorption onto Japanese eelgrass, uptake into the root zone, and aerial drift. To compare this EEC in water with empirical results, WSU researchers applied imazamox to a 30 m by 70 m sandy sediment site, 1 km north of the Port of Nahcotta (Patten and Haldeman, 2012). The treated bed was at the 2.5 feet tidal height and was covered with a moderate density of Japanese

eelgrass. The plot was treated with 16 oz/ac rate of imazamox (0.14 kg ai/ha) on May 7, 2012 using a ground-based boom sprayer, 20 minutes before the low tide (-2.6 ft). The site was dry except for a tidal drainage swale and several isolated pools. Following treatment, water samples were collected within tidal pools and swales within the treated site, in the tidal swale draining the site during the ebb tide, and on the shore side of the plot during the flood tide (**Figure 4-1**). To ensure that off-site sample locations or times of sampling occurred where and when concentrations were highest, a blue spray dye was added to the water in the outgoing drainage swale immediately after treatment, as well as to the leading edge of incoming tidal water as it moved across the site.

Figure 4-1 **Small pool within plot on the northern half of plot where imazamox was measured at 181 µg/L 3 hours before the first flood tide. (Note: no effects were observed on native eelgrass or Japanese eelgrass in the pool 21 days after treatment).**

Sampling times during the ebb tidal collection period at each collection site corresponded to times when the peak of the dye pattern was most concentrated for that location. For the 30, 60 and 120 m distances in the drainage swale, peaks occurred 9, 22 and 60 minutes after treatment, respectively. During flood tide sample collections a 5 - 8 mph northeast wind developed after spraying. Based on the dye movement patterns, this wind shifted the flow of water across the plot to the southwest. To accommodate this shift, WSU researchers moved the middle transect 30° to the south of the treated zone. Samples were typically collected at a depth of 8 cm in 2 oz, 8 cm-tall brown plastic jars provided by Sepro Labs, with a couple of samples collected in shallower water (4 or 5 cm), and the pool sample collected in water of 20 cm depth (**Table 4-1**) and then analyzed by the laboratory using EPA approved HPLC methods (1 µg/L detection limit) within 48 hours.

Table 4-1 **Water Concentrations of Imazamox following Japanese eelgrass treatment with Application Rates of 16 oz/acre (0.14 kg-a.i./acre)***

Location	Time of sample	Depth of water during sampling (cm)	Dye visible	Imazamox Detected (µg/L)
Inside swale	10:30	5	yes	541
Inside pool	10:44	20	yes	181
Ebb swale 30 m	10:39	4	yes	32
Ebb swale 60 m	10:55	8	yes	7.6

Location	Time of sample	Depth of water during sampling (cm)	Dye visible	Imazamox Detected (µg/L)
Ebb swale 120 m	11:30	5	yes	< 1
Inside 1st flood tide 3 m from outer edge (S)	12:24	8	yes	82
Inside 1st flood tide 3 m from outer edge (mid)	12:25	8	yes	61
Inside 1st flood tide 3 m from outer edge (N)	12:25	8	yes	24
Outside 1st flood tide 3 m from outer edge (S)	12:26	8	yes	79
Outside 1st flood tide 3 m from outer edge (mid)	12:27	8	yes	35
Outside 1st flood tide 3 m from outer edge (N)	12:28	8	yes	44
Outside 1st flood tide 30 m from outer edge (S)	12:46	8	yes	83
Outside 1st flood tide 30 m from outer edge (mid)	12:41	8	yes	4.7
Outside 1st flood tide 30 m from outer edge (N)	12:45	8	no	6.7
Outside 1st flood tide 60 m from outer edge (S)	13:01	8	yes	18
Outside 1st flood tide 60 m from outer edge (mid)	12:51	8	no	<1
Outside 1st flood tide 60 m from outer edge (N)	13:00	8	no	<1
Outside 1st flood tide 120 m from outer edge (S)	13:26	8	slight	5.6
Outside 1st flood tide 120 m from outer edge (mid	13:21	8	no	<1
Outside 1st flood tide 120 m from outer edge (N)	13:18	8	no	<1
Inside 2nd flood tide 3 m from outer edge (mid)	22:00	8	no	6
*Treatment applied 5/9/12 @ 9:00, tide -2.16 @ 9:19, site flood 11:55 on east edge and 12:25 on west edge.				

*Source Patten and Haldeman 2012

4.1.3 Sediment and Plant Residues

To provide data needed to reflect the anticipated sediment and plant tissue residues following treatment, a second treatment trial was conducted by WSU researchers at a 9 m by 100 m sandy sediment site, 1 km north of the Port of Nahcotta, at approximately 1.0 foot tidal height (Patten and Haldeman 2012). This site was covered with moderate to thick density of Japanese eelgrass, and was treated with 16 oz/ac rate of imazamox (0.14 kg ai/ha) on May 23, 2012 using a ground-based boom sprayer, 10 minutes after the low tide (-1.0 ft). The site was dry at application except for a few isolated small tidal drainage swales. The treated site was covered by the incoming tide between 90 to 105 minutes after treatment. Twenty four hours after treatment, with two subsequent high tides at 8.1 ft and 9.6 ft, sediment and *Z. japonica* samples were collected for tissue-residue analysis.

Sediment samples were collected using a 7-cm diameter, high density polyethylene, coring device designed to collect a clean intact sediment sample from the 0-5 cm depth. Cores were taken from sediment that was free of surface vegetation in order to obtain estimates of maximum sediment concentrations possible following treatment. A new coring device was used for each location. Five cores were collected for each of six locations at the site: two in the center south, two in the center-middle and two in the center-north. Cores were placed in Zip-lock bags and immediately placed on ice in a dark cooler. Japanese eelgrass samples were collected from the same three locations at the site.

As shown in **Table 4-2**, the average concentrations of imazamox in sediment and Japanese eelgrass tissue 24 hours after treatment, with two tidal flushes, was 5.9 and 1016 µg/kg, respectively. The maximum detected concentrations were 13 and 1500 µg/kg in sediment and Japanese eelgrass, respectively.

Table 4-2 Imazamox sediment and vegetation monitoring data*

Sample location	Imazamox (µg/kg)	
	Sediment (0-5 cm)	*Z. japonica*
South	12 & 1.9	930
Center	1.3 & 2.3	1500
North	5.2 & 13	620
Mean ± std. err.	5.9 ±2.14	1016 ± 256

*Source Patten and Haldeman 2012. (Samples triple rinsed in offsite seawater to remove associated sediment, placed in 1 gallon Zip-Lock bags, and immediately placed on ice in a dark cooler. Samples were shipped on ice within 2 hours of collection and were chemically stabilized in the lab within 24 hours and analyzed by Pacific Agricultural Labs using EPA-approved HPLC methods within 48 hours of their collection. The limit of detection was 0.5 and 100 µg/kg for sediment and vegetation, respectively).

4.1.4 Tolerance of Native Eelgrass to Imazamox

Imazamox (0.14 kg ai/ha) was applied on May 27, 2010 (-2.2 tide at 8 to 8:30 am at approximately 2.5 ft tidal height) to a 100 ft by 100 ft section of tide flats that were colonized with Japanese eelgrass (**Table 4-3**). Plots were submerged by the incoming tide at approximately 10:45 am. Within the treated area, there were small tidal pools with 5 to 15 cm mean depth of water containing patches of native eelgrass. These pools had no flowing water at the time of treatment. There were 4 pool replications for each mean depth at approximately 5, 10, 15 cm. The estimated nominal exposure concentrations for the 5, 10 and 15 cm depth pools were 278, 139 and 93 µg/L, respectively. The exposure duration prior to dilution with incoming flood water was 2.5 hours. Within each pool, stakes were placed to provide repeat measures of eelgrass shoot length at 0, 34, and 70 days after treatment. The total number of shoots measured at 0, 34, and 70 days after treatment were 230, 120, and 103 for 5 cm depth; 230, 120 and 103 for 10 cm depth, and 116, 47, and 58 for 15 cm depth, respectively.

Table 4-3 Effect of imazamox on *Zostera marina* and *Z. japonica* as a function of concentration and exposure duration*

Sample location	~imazamox exposure concentration & duration	(% control ± std. err.)	
		Z. marina	*Z. japonica*
Direct spray- no standing water	0.125 kg ai/ha – 3 hrs dry time	100±0 n=10	100±0 n=20
Direct spray or immediate runoff with <10 cm standing water	~ 400 to 550 µg/L for ~3 hrs	57 ± 6 n=16	86 ± 6 n=5
Direct spray in 20 to 30 cm standing water	~100 to 200 µg/L for 3 hours	0 n=6	0 n=6
Flood water 3 m outside of plot	35 to 80 µg/L for ~<5 to 15 minute	NA	0 n=20
Drainage swale water 6 to 60 m outside of plot	6 µg/L for ½ hr to 200 µg/L for 2.5 hrs	0 n=10	0 n=10

*Source Patten and Haldeman 2012

Native eelgrass covered by 5 to 15 cm of water was not killed when over-sprayed with imazamox. Mean shoot length of native eelgrass 34 days after treatment in the 5 and 10 cm depth pools decreased by 20 and 10 cm, respectively. By day 74, native eelgrass at 5 and 10 cm depths had grown approximately 30 and 20 cm, respectively, and both were approximately10 cm longer than at 0 days after treatment. Native eelgrass in the 15 cm pools did not show any reduction in growth 34 days after treatment and by 74 days after treatment were 30 cm longer than at 0 days after treatment. The nominal concentration of 93 µg/L could therefore be considered to approximate a NOEC for non-native eelgrass, while a LOEC of 139 µg/L could be adopted from the 10 cm

exposure depth. For conservative risk screening purposes, however, we adopted the TRV for duckweed, as summarized in **Table 3-13**.

To confirm aquatic concentrations, eelgrass stem density and percent ground coverage was also measured in 25 10-cm^2 quadrats along three north to south transects within the treated zone (summarized in **Table 4-1**). Native eelgrass coverage ranged from 0 to 20 stems/10 cm^2, with a mean of 1.9 stems. Japanese eelgrass coverage was 0 to 50 stems/10 cm^2, with a mean of 16.0 stems. Native eelgrass stem density (0.25 m^2) was also measured in marked locations (n=18, 0 to 120 m) along the drainage swale exiting the treated sites. Twenty one days after treatment, researchers assessed percent control, rating of leaf color (dark green to brown) of native eelgrass and Japanese eelgrass within and outside of the treated zone, and stem density change of native eelgrass at marked locations.

At the time of treatment, the percent of ground coverage by both eelgrass species ranged from 2% to 95% with a mean of 20%. Thirty days after treatment, the percent on-site Japanese eelgrass control was 100% (**Table 4-3**). The off-site Japanese eelgrass control on the flood side of the treatment zone was zero. Similarly, there were clean lines differentiating treated and untreated locations on the south and north of the plot.

The fate of native eelgrass sprayed within the treated zone in this trial varied by location. Native eelgrass not covered by water (i.e., not in the drainage swale) was 100% affected (eliminated). Native eelgrass directly sprayed but covered by < 10 cm of standing pooled water was reduced by 57%, and there was no effect on native eelgrass if it was covered by 20 to 30 cm of water. There was no measured effect of imazamox on native eelgrass in the drainage swale beyond 6 m from the treated zone.

Based on the data provided in **Table 4-3**, native eelgrass may be slightly more tolerant of imazamox than is Japanese eelgrass. However, data are not sufficient to fully assess this outcome. Therefore, it is concluded that native eelgrass is equally susceptible at the concentrations that would be applied to Japanese eelgrass

4.1.5 Summary of Environmental Exposure Concentrations

Table 4-4 summarizes the maximum and average concentrations detected in environmental media sampled for the proposed treatment program with imazamox, based on the field trials summarized in **Sections 4.1.1** through **4.1.3**.

Table 4-4 Summary of Maximum and Average Imazamox Detections in Relevant Environmental Media with Application Rates of 16 oz a.i./acre

Environmental Media		Average of Maximum Replicate Location Measured	Average of All Samples Measured,
Surface Water	541 µg/L	55.67 µg/L @ 3 m from outer edge	29.8 µg/L (all samples inside and outside from 3 m to 120 m)*
Treated Sediment	12 µg/kg	9.1 µg/kg @ north site (see Table 4-2)	5.9 µg/kg
Plant Tissue (*Z. japonica*)	1500 µg/kg	NA (no location replicates taken)	1016 µg/kg

*non-detects counted at detection limit, all data from tables 4-1 and 4-2.

4.2 ECOLOGICAL RECEPTOR EXPOSURE

Specific doses are estimated for imazamox to terrestrial and aquatic receptors, based on exposure factors representative of the species' life histories. Life history data for ecological receptors considered in this assessment were provided in **Section 2**. The species modeled for exposure (or closely related species) exhibit habits or live in habitats where Japanese eelgrass is distributed and exposure could occur, and/or they are test species for which toxicological data have been developed, and/or they are species of particular concern to the public. Relevant exposure factor information for mammalian, avian, amphibious, reptilian, aquatic wildlife, and nontarget terrestrial invertebrates, to the extent it could be identified, is summarized for the receptors considered in this SLERA in the following sections. These factors were derived from a variety of sources, including the Wildlife Exposure Handbook (EPA 1993), references summarized on the Ecotox database (http://oehha.ca.gov/cal_ecotox/), or primary literature (as cited). Average weights, surface areas, and daily consumption rates were used to represent exposure to receptors. These numbers can exhibit a great deal of variation among populations, but addressing population-specific data for ecological receptors in each of the areas where Japanese eelgrass is distributed and treatments could occur was well beyond the scope of this report.

The doses received by each receptor are a function of exposure factors specific to the species and/or guilds modeled, and are discussed in this section by guild. Based on conceptual exposure considerations and empirical results from site-specific testing described in **Section 4.1**, only ingestion is considered for mammalian and avian receptors. Dermal contact and inhalation are not estimated because the method of application and the location of application would be extremely unlikely to contribute significant additive doses of imazamox to the mammalian and avian receptors evaluated. Further, the use of the NOEC or NOEL as the TRV provide for significant conservatism to incorporate any marginally additional dose contributed by these pathways. However, ingestion includes exposure via several possible media—food, sediment and water. To consider the cumulative exposure by each of these media, **Equation 2-1** is expanded as indicated in **Equation 4-1** to calculate the total daily intake (TDI), wherein:

Equation 4-1	**Total Daily Intake** $$TDI = (Csl \times SIR) + \sum_{i=1}^{n} (Ci \times Pi \times FIR) \times 1/BW \times AUF$$ **= Total Dose acquired from sediment and dietary items**

Where:

TDI	=	total daily intake (milligram per kilogram body weight per day or mg/kg-day)
Csl	=	maximum concentration in soil (milligram per kilogram or mg/kg)
SIR	=	sediment ingestion rate (kilogram per day or kg/day)
Ci	=	concentration in each dietary item (mg/kg)
Pi	=	fraction of diet as item i (unitless)
FIR	=	food ingestion rate (kg/day)
BW	=	body weight (kg)
AUF	=	area use factor

It was conservatively assumed that all receptors would derive 100% of their food and drinking water and 100% of their incidental ingestion of sediment from the treatment area. That is, an area use factor (AUF) of 1 was used regardless of the actual life histories and home range areas of the receptors. For the ground applications proposed, this practice likely overestimates exposure significantly, although it is the appropriate first step for SLERAs.

The cumulative daily intake by all ingestion pathways was used to develop HQs (**Table 4-5**). For screening, this assumption presumes that ingestion uptake from each environmental media (i.e., diet, water, sediment) could elicit the same effect through the same mechanism of action. That is, toxicological or pathological outcomes from exposure will be dose neutral regardless of pathway.

4.2.1 Wildlife Ingestion Exposure Estimation

Exposure factors considered for modeling in wildlife are provided in **Table 4-5**. Exposure via ingestion was determined from the empirical water, sediment, and plant residue studies discussed in **Section 4.1**, and summarized in **Table 4-4**. The maximum concentrations in each medium measured were used as input variables to estimate acute doses. The average of the maximum values measured at a discrete location after treatment was used to estimate subchronic exposure conditions. The average of all samples measured was used to estimate the chronic EECs to which ecological receptors could be exposed.

Table 4-5 Exposure Parameters for Mammalian and Avian Wildlife for Addressing Risks from Imazamox Applications to *Zostera japonica*

Species and Status	Adult Body Weight (g)	Food Intake (g/day)	% Vegetation in Diet	% Animal Matter in Diet	Soil and Sed Intake (% of diet)	Water Intake (mL/day)
Bobwhite quail	174	13.5			9.3	19
Marsh wren	11.25	8	0.05	0.95	2.1**	3
Mallard	1,170	420	0.6	0.4	3.3	65
Cottontail rabbit	1,200	79	100	0	6.3	116
Norway rat	300	15	0.5	0.5	2	33
Coyote*	9,800	449	0.25	0.75	2.8	871

Source: EPA 1993 or Sample et al. 1997, unless noted by an asterisk

*Exposure factors marked by an asterisk were calculated using allometric formulae in the "Wildlife Exposure Factors Handbook" (EPA 1993); **converted from quail ratio

Exposure factors were obtained from the Wildlife Exposure Handbook (EPA 1993) or Sample et al. (1997), as presented in **Table 4-5**. We used soil consumption rates as a proxy for sediment consumption for wildlife, which likely overestimates sediment consumption for many of the non-water dependent species considered. Acute, subchronic, and chronic ingestion exposure doses of imazamox in avian and terrestrial wildlife are summarized in **Table 4-6**.

Table 4-6 Estimated Cumulative Ingestion Exposures to Terrestrial Wildlife Receptors from Imazamox Applications (mg/kg-body wt)*

Receptor	Acute Ingestion Exposure Dose	Subchronic Ingestion Dose	Chronic Subacute Ingestion Dose
Mallard	0.569	0.369	0.367
Bobwhite Quail	0.176	0.086	0.083
Marsh Wren	1.211	0.731	0.737
Coyote	0.117	0.049	0.052
Cottontail Rabbit	0.151	0.073	0.070
Norway Rat	0.135	0.057	0.054

4.2.2 Insignificant Wildlife Exposure Pathways

Additional routes of potential exposure are acknowledged both in the conceptual model (**Figure 2-1**) and also in **Table 4-2**. However, ingestion pathways are assumed to be both likely and maximal. Chronic exposure to contaminated water is likely insignificant because of the intertidal conditions where the applications would occur. Estimated chronic exposure to an assumed water concentration collected as the TRV for chronic exposure (0.030 µg/L) overestimates what would be available to wildlife receptors after two or more tidal exchanges. Thus, the chronic doses based on food, sediment, and water intake, adequately account for minor pathways that are not quantitatively evaluated. The water intake input parameters presumed drinking of herbicide contaminated freshwater, which would be unlikely in the estuarine application area for most of the species modeled. However, because waterfowl have salt glands they are able to drink saltwater.

Acute inhalation exposure is possible, but because it is considered insignificant it was not quantitatively modeled. The low significance of the exposure pathway is related to the disturbance created during treatment that causes mobile animals to avoid the area being immediately treated and thereby avoid drift exposure.

4.2.3 Aquatic Exposures

This SLERA evaluates two types of aquatic receptors: fish and aquatic invertebrates. Water-dependent mammals and birds are considered within the terrestrial exposure factors. Fish and aquatic invertebrates could be exposed to imazamox through direct application and mixing within the water bodies that support them. The maximum concentrations in water summarized in **Table 4-4** were used as input variables to estimate acute exposure. The average of the maximum values measured at a discrete sample location was used to estimate subchronic aquatic exposure conditions, and the average of all samples measured was used to estimate the chronic water concentration to which fish and aquatic invertebrates might be exposed.

As indicated in the CSM for aquatic animals (**Figure 2-2**), aquatic receptors may be exposed to imazamox via ingestion. However, this pathway was not quantitatively evaluated for fish or aquatic invertebrates due to substantial uncertainty in the concentrations of imazamox in the diet of fish and the lack of dietary TRVs. As imazamox does not bioconcentrate or bioaccumulate, significant dietary exposure to higher trophic level receptors is highly unlikely. Finally, EPA toxicity criteria are based only on aquatic exposure through water, which essentially integrates multiple exposure pathways (diet, gill transfer, direct contact).

The maximum concentrations in water summarized in **Table 4-4** were used as input variables to estimate acute exposure. The average of the maximum values measured at a discrete sample location were used to estimate subchronic aquatic exposure conditions, and the average of all samples measured were used to estimate the chronic water concentration to which fish and aquatic invertebrates might be exposed. These concentrations were compared to the aquatic TRVs listed in **Table 4-4** to characterize risks in **Section 5**.

5 Risk Characterization

The purpose of risk characterization is to integrate the findings from preceding sections in order to estimate the likelihood, severity, and spatial extent of any predicted adverse effects from use of imazamox. Hazard quotients for imazamox to ecological receptors describe the potential adverse effects posed by the proposed herbicide treatment. Hazard quotients were calculated by dividing the EEC by the TRV. Interpretation of HQs reflected the following guidelines.

- **Non-listed Species Direct Acute Hazards:** Significant hazards are associated with a HQ greater than 0.5 for aquatic animals, which implies that greater than 50% of laboratory test organisms exposed to the peak EECs would be expected to exhibit an effect greater than or equal to that described by the standard acute toxicity endpoint.
- **ESA- listed Species Direct Acute Hazards:** Significant hazards are associated with a HQ greater than 0.05 for aquatic animals, which implies that greater than 5% of the laboratory test organisms exposed to the peak EECs would be expected to exhibit an effect greater than or equal to that described by the standard acute endpoint.
- **Direct Chronic Hazards for ESA:** Significant hazards are associated with a HQ greater than 1 for listed and non-listed animals, which implies that the long term EECs would be greater than or equal to that described by the standard acute endpoint.
- **Non-listed Aquatic and Terrestrial Plant Direct Hazards**: Significant hazards are associated with a HQ greater than 1, which implies that 100% of the laboratory test organisms exposed to the EECs would be expected to exhibit an effect greater than or equal to that described by the standard endpoint for non-listed species.
- **ESA-listed Aquatic and Terrestrial Plant Direct Hazards:** For listed aquatic and terrestrial plant direct effects, significant hazards are associated with a - HQ greater than 1, which implies that 100% of the laboratory test organisms exposed to the EECs would be expected to exhibit an effect greater than or equal to that described by the standard endpoint for listed plant species.

Because safety factors of at least 10-fold were applied to TRVs in test species for nontest species, T&E species hazards were appropriately considered.

5.1 ECOLOGICAL RECEPTOR RISK CHARACTERIZATION AS ESTIMATED BY HAZARD QUOTIENTS

Potential hazards to ecological receptors are a function of two equally important factors: 1) duration of exposure and 2) the concentration of imazamox. Hazard quotients (HQs) were derived to consider acute, subchronic, and chronic exposure. The following HQs, derived from exposures estimated in **Section 4** and the TRVs listed in **Table 4-4**, are presented below for avian and terrestrial wildlife, and aquatic animals and plants.

5.1.1 Avian and Terrestrial Wildlife Risk

Table 5-1 presents acute, subchronic, and chronic HQs for wildlife receptors. No significant hazards are predicted for ESA-listed and/or non-listed in terrestrial species. Wildlife hazards are therefore insignificant.

Table 5-1 Hazard Quotients to Avian and Terrestrial Wildlife from Potential Imazamox Exposure

Receptor	Acute Ingestion Exposure Hazard Quotient	Subchronic Ingestion Dose Hazard Quotient	Chronic Subacute Ingestion Dose Hazard Quotient
Mallard	0.0001	0.0002	0.0002
Bobwhite Quail	0.00003	0.00004	0.00005
Marsh Wren	0.0004	0.0005	0.0005
Coyote	0.0001	0.00004	0.00004
Cottontail Rabbit	0.00004	0.0002	0.0002
Norway Rat	0.00003	0.0001	0.0001

5.1.2 Aquatic Animal and Plant Hazard Quotients

The hazard quotients for aquatic organisms are presented in **Table 5-2**. The subchronic NOEC was conservatively assumed to be equal to the chronic NOEC for these calculations. As demonstrated in **Table 5-2**, risks to fish and aquatic invertebrates are predicted to be insignificant from the proposed program. None of the hazard quotients calculated using conservative input parameter data and the NOEC values (rather than LC_{50}s) exceed levels of concern. Risks to non-target plants could be significant, based on a comparison to the effective control concentration for duckweed (**Table 4-4**), and as supported by the field studies with eelgrass conducted in Willapa Bay. These findings are summarized in **Section 4** and are detailed in Appendix A.

Table 5-2 Hazard Quotients to Aquatic Animals From Potential Imazamox Exposure

Receptor	Acute Aquatic Exposure Hazard Quotient	Subchronic Aquatic Exposure Hazard Quotient	Chronic Aquatic Exposure Hazard Quotient
Rainbow Trout	0.0044	0.0047	0.0025
Dungeness Crab	0.0057	0.0059	0.0032
Vascular Plants (duckweed/eelgrass, etc.)	49	51	27

*Risk quotient defined as: EEC/NOEC (animals) or EEC/EC_{50} (plants)

5.1.2.1 Non-target Aquatic Vegetation Risk

Risks to non-target aquatic vegetation represent the most significant risks associated with the use of imazamox. This finding is expected, given that the herbicide was designed to be a broad-spectrum agent to control unwanted plant growth. Risks to algae (marine diatoms, Ulva, etc.), based on testing that failed to generate an effect at the anticipated EEC, are insignificant. In contrast, risks to vascular plants such as native eelgrass may be significant, based on the expected water concentrations and EC_{50} values for duckweed, a floating vascular plant. Hazard quotients exceeded 1 under each of the exposure scenarios considered for vascular plants and using the TRVs established under static lab conditions with duckweed.

The impact of imazamox use on native eelgrass would appear to be largely controllable through implementation of the proposed buffer of 10 m where the two species can overlap in the lower portions of Japanese eelgrass distribution. Field monitoring of effects on native eelgrass showed no effect to the native grass species 6 m from the spray zone (**Table 4-3**). The 10 m buffer proposed provides a margin of safety nearly double the distance where no effect to the native species was recorded, and monitoring proposed under the draft Ecology permit should allow for refinement of this buffer, as necessary.

5.2 UNCERTAINTIES AND DATAGAPS

Uncertainties in the estimation of exposure and selection of TRVs are inherent to all SLERAs. Conservative assumptions are customarily adopted in order to compensate for such uncertainties. These conservative assumptions result in overestimation of the likelihood of adverse effects. This practice ensures that risks are not underestimated at the screening level. In this assessment the principal uncertainties and sources of conservatism include:

- Hazards from imazamox were estimated where the toxicological testing conducted did not produce significant adverse health effects in test animals and no dose response could be generated. Under these circumstances, it might be argued that the use of NOAEC and NOAEL TRVs for risk screening lacks foundation, as these values are appropriately set empirically when a dose response relationship can be modeled or extrapolated from an established LC_{50} or LD_{50}. Yet, as part of the screening process, to be consistent in the analysis across all receptors modeled, it was necessary to project TRV values based on the NOELS, even though they did not yield adverse health outcomes and no LD_{50}s could be developed. The outcomes of such modeling can lead to erroneous conclusions of potential risk, wherein little or none exists, simply because the animal modeled has, for example, a high intake rate (e.g., marsh wren) and/or the TRV NOAEC value was set at a low value based on screening guidance for the application of safety factors, regardless of whether a toxic mechanism of action could occur in the animal, or whether they would be in the area of treatment for sufficient duration to assimilate exposure. Based on the paradigms applied, safety factors can lower the TRV value from a tested species by over two orders of magnitude, which can and likely does result in the potential overestimation of risk quotients, for all treatment chemicals considered.
- In none of the animal tests conducted were doses identified that elicited conclusive toxicity or pathogenicity with imazamox. Thus, the HQ can be overestimated by presuming a NOAEL that is too low even before safety factors are applied, which is compounded with safety factors that are applied to the NOAEL for species that are unrelated.
- Some species will not inherently use areas that will be typically treated (e.g., rabbit), yet their diet was presumed to be equally composed of treated forage as that of the duck, for example. Through risk screening, this approach tends to maximize the dose received, which can subsequently affect the hazard quotient calculation.
- The acute water concentrations used to characterize risks from drinking (ingestion component) and aquatic exposure were based on the maximum concentration detected in field testing. This concentration exceeded the theoretical maximum concentration at full dilution, with the proposed application rate. This may reflect localized tidal draining and concentration from the treatment area into swale waters. The use of the maximum concentration detected empirically, as opposed to using the nominally anticipated water concentration was appropriately conservative, however, as it reflected a more ‘real world’ scenario. Notwithstanding, this maximum concentration did not suggest risks to non-target macroalgae or animals would result from ingestion or aquatic exposure. Aquatic fate, particularly in regularly circulating tidal waters, will rapidly reduce any bioavailable concentrations of applied treatment chemicals, such that the static concentrations presumed would be unlikely to be experienced by aquatic receptors. Therefore, the acute and chronic hazard quotient estimations for aquatic receptors, based on aquatic waterborne exposure, likely exaggerate risk.
- The area to be treated is significantly large and it was not possible, without an extensive research project, to consider specific habitats wherein highly localized ecological receptors might live and be exposed (or avoid exposure). As a result, area use factors of 100% were

assumed for all species modeled. Highly mobile receptors, will avoid significant exposure by moving out of the immediate treatment area. The presumption that receptors will be equally exposed throughout their entire home range increases the estimated dose, and hence, the estimated hazard quotient.

- Inhalation toxicity values were not available for many ecological receptors from which to compare against estimated inhalation doses for those animals where intake rate information was available. This lack of information represents a data gap in virtually all ecological risk assessments. Regardless, inhalation exposure was not considered to represent significant additive dose in the present assessment because the method of application (ground-based boom sprayer) would yield limited drift. Therefore, the lack of an estimate on inhalation dose is not anticipated to materially alter the risk characterization conclusion for those ecological receptors, particularly given other conservative calls that were made in considering risks (e.g., use of NOELs as the TRVs, assumed site use of exposed areas for 100% of the life history of the modeled animals, etc.).
- Surface contact can contribute to cumulative exposure from ingestion and inhalation through transdermal uptake and preening; however, the feather and fur barrier substantially reduces the potential additive exposure through this pathway. Although surface contact was recognized as a potential exposure pathway, the hazards from the additive dose potential from this pathway was not considered significant given the intertidal application proposed, and the disturbance that will be created by applicators that will limit such contact to animals. The application of safety factors applied to the ingestion doses provide more than sufficient conservatism to account for any additive dose through surface contact that would otherwise occur and would be extremely unlikely to alter risk conclusions.

5.3 CONCLUSIONS

This SLERA finds that risks are not significant for non-target fish, invertebrates, wildlife, and macroalgae as a result of the use of imazamox to control Japanese eelgrass. Risks to non-target vascular plants, particularly native eelgrass, could be significant in the absence of measures to minimize impacts to this species. Use of the proposed buffers to avoid unnecessary impacts to native eelgrass should provide sufficient margin of safety to minimize impacts to native eelgrass. Further monitoring, as outlined in the Ecology draft permit, will enable adaptive management refinement, if needed.

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Appendix A

Imazamox efficacy studies on Japanese Eelgrass

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Japanese Eelgrass Control with Imazamox

Trial ID: EELGRASS 1 2006 Location: STACKPOLE Investigator: Kim Patten

Treatment Name	Rate	Rate Unit	% Japonica control top growth compared to untreated adjacent ground 4/4/07	% Japonica coverage of tide flat compared to untreated adjacent ground 6/27/07
CLEARCAST- PINK	16	fl oz/a	86.0 a	74.7 a
COMPETITOR	1	qt/a		
CLEARCAST- ORANGE	32	fl oz/a	91.7 a	70.0 a
COMPETITOR	1	qt/a		
CLEARCAST- BLACK	64	fl oz/a	97.7 a	53.3 a
COMPETITOR	1	qt/a		
LSD (P=.05)			15.67	19.28
Standard Deviation			6.91	8.50
CV			7.53	12.89
Replicate F			3.079	12.290
Replicate Prob(F)			0.1551	0.0196
Treatment F			2.137	5.217
Treatment Prob(F)			0.2337	0.0768

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Treatment information: Applied 9/20/06 @ 8 am with high tide @ 1:30 – plots covered ~ 3 hours following treatment.
Air 60 f, soil 60 f, wind 1-2 mph s, % cloud cover 100%, 3 replications, 12'x20' plots, 30 gpa spray volume 5 nozzle boom, site was thick silt with very thick coverage with japonica tidal ht ~ 3-4'.
Assessment information: 10/25/06 no real visual effects; 4/4/07 – big treatment effect; 6/27/07 big treatment effect. By 2008 treatment effect gone (no data).

Summary: Higher rate required for good control when applied in fall on fully established bed of japonica. Treatment effect held for most of year, but then gradually disappeared by summer 2007 and summer 2008. This was due to re-establishment from new seedlings spring 2007.

Late Spring Japanese Eelgrass Control with Imazamox on Clam Beds

Trial ID: EELGRASS 2 2007 Location: Stackpole Investigator: Kim Patten

Treatment		% Japonica control top growth 6/27/07	% Japonica control top growth 9/14/07	% Bare ground 9/14/07	Japonica top dry wt grams /0.25m^2 12/5/07	% Japonica coverage of ground by canopy 10/14/08
IMAZAMOX COMPETITOR	8 fl oz/a 1 qt/a	65.0 b	65.0 a	70.0 a	22.0 b	100.0 a
IMAZAMOX COMPETITOR	16 fl oz/a 1 qt/a	65.0 b	55.0 a	81.7 a	34.4 b	100.0 a
IMAZAMOX COMPETITOR	32 fl oz/a 1 qt/a	95.3 a	91.7 a	90.7 a	13.6 b	100.0 a
IMAZAMOX COMPETITOR	64 fl oz/a 1 qt/a	99.3 a	99.0 a	97.7 a	0.9 b	100.0 a
control					87.0 a	100.0 a
LSD (P=.05)		20.52	41.92	24.07	39.91	0.00
Standard Deviation		10.27	20.98	12.05	20.67	0.00
CV		12.66	27.02	14.17	65.44	0.0
Replicate F		3.185	3.324	2.487	2.083	0.000
Replicate Prob(F)		0.1141	0.1067	0.1635	0.1950	1.0000
Treatment F		9.983	3.010	2.955	7.801	0.000
Treatment Prob(F)		0.0095	0.1163	0.1200	0.0102	1.0000

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Treatment information: applied 5/3/07; 10'x35' plots, 3 replications, thick soupy silt ground @ 3' tidal ht, eelgrass just beginning to grow- still fairly thin, plots covered with some water at application 0-1", covered in 1-2 hours post treatment.

Assessed: 6/27/07, 9/14/07, 12/5/07 and 10/14/08.

Summary: Medium rate 32 oz/ac required for best control when applied in marginal area, soppy, messy. Treatment effect held for 1 year, but then gradually disappeared by summer and fall 2008. Results suggest a need for annual treatment in order to maintain year-round japonica-free tideland.

Native Eelgrass (*Z. marina*) Control with Imazamox

Trial ID: EELGRASS 3 2007 Location: NAHCOTTA Investigator: K Patten

Treatment Name	Rate	Rate Unit	Marina % CONTROL 9/26/06	Japonica % CONTROL 9/26/06	Marina % COVER 9/26/06	Japonica % COVER 9/26/06
CLEARCAST	16	fl oz/a	94.3 a	94.7 a	7.0 b	3.7 b
COMPETITOR	1	qt/a				
CLEARCAST	32	fl oz/a	99.0 a	97.7 a	2.7 b	2.0 b
COMPETITOR	1	qt/a				
CONTROL			0.0 b	0.0 b	90.0 a	90.0 a
LSD (P=.05)			10.12	11.55	14.85	6.90
Standard Deviation			4.47	5.09	6.55	3.05
CV			6.93	7.94	19.73	9.55
Replicate F			1.376	0.955	1.105	1.593
Replicate Prob(F)			0.3509	0.4581	0.4150	0.3099
Treatment F			469.343	356.715	169.229	819.174
Treatment Prob(F)			0.0001	0.0001	0.0001	0.0001

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Application notes: Applied 6/1/07; 3 replications, 10' x 12' plots, wind 5 mph NW. Temp 55, 100% overcast, no rain, tide covered in 2 hours following treatment. Sandy site with both species @ 1-2' tidal ht. Rep 1 mostly submerged, rep III mostly dry, rep II in between.

Assessed: 9/27/09 based on % control and % coverage, rep three most control, rep 1 the least.

Summary: Native eelgrass equally sensitive to imazamox as *Z. japonica*. Native eelgrass avoids treatment effect if submerged.

Spring Japanese Eelgrass Control with Imazamox on Clam Beds

Trial ID: EELGRASS 1 2008 Location: Stackpole Investigator: Kim Patten

			Japonica % coverage of ground 10/14/08		Marina % coverage of ground 10/14/08	
Treatment Name	Rate	Rate Unit	mean	StErr	mean	StErr
IMAZAMOX	25	fl oz/a	18.9	3.2	0.0	0.0
COMPETITOR	0.45	qt/a				
IMAZAMOX	35	fl oz/a	28.3	5.8	7.3	3.6
COMPETITOR	0.75	qt/a				
UNTREATED CHECK			98.0	1.4	30.7	9.7

Application information: Applied 5/6/08; 1 replication of 12 x 300' @ 9am light mist 100% overcast, 49 F, wind 0, japonica sparse at application with 10 to 40 plants/m^2 leaves 2-6" long, < 10% ground coverage. Only a few native eelgrass plants on site, except for channel. Silty muddy site, a drainage channel in middle of plots with native eelgrass totally covering channel at application time.

Assessment information: assessed for visual coverage of ground and control on 10/14/08, multiple assessments within each treatment to get standard error. By 2009 ground mostly covered with japonica, dry wt data available but needs to be entered into data set. Drainage channel with *marina* unaffected by treatment – 100% coverage before and after.

Summary: Native eelgrass equally sensitive to imazamox as *Z. japonica*. Native eelgrass in channels unaffected by treatment. Some japonica from late germinating seeds came into plots post-treatment. Treatment effects lasted ~ 1 year.

Spring Japanese Eelgrass Control with Imazamox on Clam Beds

Trial ID: EELGRASS 2 2008 Location: LEADBETTER- Sheldon Investigator: Kim Patten

Treatment		Japonica % control 6/17/08 Dry site	Japonica %control 6/17/08 Slightly Wet site	Marina % control 6/17/08 Low spots/ slightly wet sites within plots	Japonica % coverage 10/14/08	Marina % coverage 10/14/08
IMAZAMOX	11.5 fl oz/a	98.0	50.0	10.0	16.4	8.8
COMPETITOR	1 qt/a					
IMAZAMOX	23 fl oz/a	100.0	96.0	80.0	7.4	0.0
COMPETITOR	1 qt/a					
IMAZAMOX	34 fl oz/a	100.0	100.0	90.0	9.0	2.6
COMPETITOR	1 qt/a					
IMAZAMOX	45 fl oz/a	100.0	100.0	90.0	12.2	4.8
COMPETITOR	1 qt/a					
UNTREATED CHECK		0.0	0.0	0.0	68.2	30.5

Application information: applied 5/6/08, 8:30 am, single replication of 12' by 100', light mist at end of application, 100 % overcast, wind 10 to 20 mph NW, 49 F, tide -2.9 at 9 am, plots dry at application except for low spots containing native eelgrass. Marina at these sites was mostly submerged in 1-4" water (pools), Here were also some low spots of Zostera japonica. These low populations of native eelgrass were most submerged with little leaf material exposed. Japonica was sparse with 20 to 40 plants per m^2, leaves, 2 to 6" long, < 10 % ground coverage. Japonica was dry (most locations) at time of application.

Assessment information: plots assessed 6/17/08 and 10/14/08 for control and/or coverage. Data taken for wet and dry sites separately. Eelgrass considerable thicker at assessment than application.

Summary: With good treatment conditions efficacy was achieved at 11.5 oz/ac (dry and early). The low rate of imazamox lost efficacy when a little water was over the canopy. Marina within site was controlled with imazamox at 23 oz/ac if leaves were exposed. By end of season treatment effects held, but by 2009 seedlings were covering plots.

May Mixed Invasive Seagrasses Control with Imazamox on Clam Beds - Large Plots 2008

Trial ID: EELGRASS 5 2008 Location: Leadbetter Investigator: Kim Patten

Treatment		Japonica % coverage 10/14/08 Site 1	Japonica % coverage 10/14/08 Site 2
UNTREATED CHECK		84.6	94.0
IMAZAMOX AGRIDEX	12 fl oz/a 1 qt/a	2.6	1.5
IMAZAMOX AGRIDEX	24 fl oz/a 1 qt/a	0.0	0.9
IMAZAMOX AGRIDEX	48 fl oz/a 1 qt/a	0.0	2.2
IMAZAMOX AGRIDEX	75 fl oz/a 1 qt/a	0.0	0.3

Application comments: site one applied May 27 2008, 1 replication 11' by 250' , 2-3 hr dry time applied @ 12:45; site two applied May 28 1:30 to 2:00, 1 replication 22 by 200', japonica density, thin 2-4" long, Temperature both days 54, wind 510 NW, 100% overcast, no rain, plot tidal height 3.5'

Assessed: 10/14/08 for % coverage by japonica and plots look real good.

Summary: Low rate of imazamox effective when conditions are right. Treatments held up until midsummer 2009.

May Mixed Invasive Seagrass Control with Imazamox on Clam Beds 2008

Trial ID: EELGRASS 6 2008 Location: Leadbetter Investigator: Kim Patten

Treatment		Japonica % coverage 10/14/08 treated zone	Japonica % coverage 10/14/08 adjacent Untreated zone	% exposed gravel and shell 10/14/08 treated zone	% exposed gravel and shell 10/14/08 adjacent Untreated zone
IMAZAMOX +	12 fl oz/a	0.0 a	46.0 a	42.0 a	0.7 a
COMPETITOR	1 qt/a	0.0 StErr	6.3 StErr	9.0 StErr	0.7 StErr
IMAZAMOX +	24 fl oz/a	0.0 a	59.0 a	44.2 a	0.0 a
COMPETITOR	1 qt/a	0.0 StErr	11.0 StErr	10.9 StErr	0.0 StErr
IMAZAMOX +	48 fl oz/a	0.0 a	62.7 a	60.0 a	0.0 a
COMPETITOR	1 qt/a	0.0 StErr	11.2 StErr	9.4 StErr	0.0 StErr
IMAZAMOX +	96 fl oz/a	0.0 a	87.3 a	53.0 a	0.0 a
COMPETITOR	1 qt/a	0.0 StErr	7.5 StErr	9.4 StErr	0.0 StErr
LSD (P=.05)		0.00	30.67	53.86	1.15
Standard Deviation		0.00	17.72	25.66	0.58
CV		0.0	27.8	51.53	346.41
Replicate F		0.000	7.778	0.217	1.000
Replicate Prob(F)		1.0000	0.0172	0.8118	0.4219
Treatment F		0.000	3.799	0.314	1.000
Treatment Prob(F)		1.0000	0.0772	0.8153	0.4547

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Application comments: Applied 5/28/08 11:15 to 1:30, 5 replications, 11'x11' plots, on gravel with thin *Z. Japonica,* and thick *Ulva intestinalis, Polysiphonia hendryi var. Deliquescens, Ulva flexuosa.* temp 54, wind 5- 10 NW, 100% overcast - no rain, water off @ 11, back on at 4, tide ht of plot ~ 3.5 to 4', plots all had rock with the above algae attached.

Assessed: Data collected 6/17/08 on macroalgae and 10/14/08 on eelgrass, good data, all treatment controlled japonica, no treatment controlled the other species

Summary: Results indicate late May treatment very effective on japonica at low rates. Imazamox had no effect on *Ulva intestinalis, Polysiphonia hendryi var. Deliquescens,* or *Ulva flexuosa.*

Imazamox Rate for Spring Eelgrass Control 2009

Trial ID: eelgrass 2 2009 Location: Sherwood Investigator: Kim Patten

Treatment 6		% control japonica 5/29/09
IMAZAMOX	4 fl oz/a	98.0 a 1.5 StErr
IMAZAMOX	8 fl oz/a	99.7 a 0.3 StErr
IMAZAMOX	12 fl oz/a	99.7 a 0.3 StErr
IMAZAMOX	16 fl oz/a	99.7 a 0.3 StErr
IMAZAMOX	24 fl oz/a	99.7 a 0.3 StErr
control		0.0 b 0.0 StErr
LSD (P=.05)		1.68
Standard Deviation		0.92
CV		1.12
Replicate F		4.740
Replicate Prob(F)		0.0356
Treatment F		5768.052
Treatment Prob(F)		0.0001

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Application comments: applied 4/27/09 @ 8-820 am, 12' x 12' 3 replications, 30% japonica coverage (thin) jeg cover temp ~ air 47 f, Overcast 100 %, soil temp ~ 54, sunny by 10:30, wind 1-3 NW, most mature japonica with some seedlings 6-8" long.
4' tidal ht, bare sand

Assessed: 5/29/09 – everything controlled, except a few wet spots, reassess later in summer with no change in control – still 100% (no data taken)

Summary: Under perfect conditions – small plants, good dry time and dry site efficacy was obtained at 4 oz/ac.

Imazamox Rate for Summer Eelgrass Control -Eelgrass 3 2009

Trial ID: eelgrass 3 2009 Location: Sherwood Investigator: Kim Patten

Treatment		% of canopy browned down 6/26/09	% control 7/7/09
IMAZAMOX	4 fl oz/a	76.0 a	91.7 a
IMAZAMOX	8 fl oz/a	96.3 a	98.3 a
IMAZAMOX	12 fl oz/a	97.7 a	99.7 a
IMAZAMOX	16 fl oz/a	98.3 a	100.0 a
IMAZAMOX	24 fl oz/a	97.7 a	99.3 a
control		0.0 b	3.3 b
LSD (P=.05)		18.63	8.59
Standard Deviation		10.24	4.72
CV		13.18	5.75
Replicate F		0.765	1.805
Replicate Prob(F)		0.4909	0.2142
Treatment F		43.557	201.496
Treatment Prob(F)		0.0001	0.0001

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Application comments: applied 6/10/09 @ 9:35 to 9 45, 3 replications 11' x11', 100% overcast. wind 0, temp 55, tidal ht ~ 2.5', covered at ~ 1:30. Thick uniform stand of japonica at time of application.

Assessed: 6/26/09 for % of canopy browned down and 7/7/09 for % control

Summary: Under perfect conditions – small plants, good dry time and dry site efficacy was obtained at 4 to 8 oz/ac.

Appendix B

Stomach Content Analysis of Ducks Shot in Willapa Bay WA during Fall and Winter 2009 and 2010.

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Introduction

The expansion of Japanese eelgrass in Willapa Bay has caused concern in the commercial shellfish industry. Infestations of Japanese eelgrass can cause major reductions in hard shell clam production (Fisher & Patten 2011). The recent listing of Japanese eelgrass as a Class C noxious weed in Washington has alarmed waterfowl hunters who fear that significant loss in foraging habitat could occur if Japanese eelgrass control commences. To address these concerns, a study was conducted in 2009 and 2010 to try to quantify the level of waterfowl foraging on Japanese eelgrass in Willapa Bay.

Methodology

During the hunting season of 2009 and 2010 esophagus and proventriculus contents of 118 duck samples were collected from hunters in Oysterville, Nahcotta, Porter Point Willapa National Wildlife Unit, and Nemah Flats. A total of 18 mallard (*Anas platyrhynchos)*, 66 pintails (*A. acuta)*, 14 teal (*A. carolinensis)* and 20 wigeon (*A. americana)* were collected. The gizzards were separated from proventriculi prior to examination. The upper gastrointestinal tract was dissected lengthwise from the proximal proventriculus sphincter to the distal end of the esophagus. GI tissue was opened and laid out flat to expose contents of the tract. A dissecting microscope was used as an aid to separate contents from tissue, and to separate samples of animal, mineral and vegetable volumes. Separated contents were air dried and weighted. When possible (if distinguishable) the number of Japanese eelgrass leaf blades was counted in each bird.

Results

A total of 118 ducks across 4 species were sampled (Table 1). Pintail foraged the least on Japanese eelgrass (15%) and Wigeon (85%) the most. Pintail and Teal had only trace amounts of Japanese eelgrass in their stomachs. Mallard had the highest level of foraging on Japanese eelgrass seeds of the 4 species. None of the mallards obtained on the refuge contained Japanese eelgrass. Many (approximately 1/3rd) of the duck samples had empty esophagi and proventriculi. Overall, the total dry amount of Japanese eelgrass contained within Mallards, Pintails and Teals was fairly insignificant (<0.1 g/bird), while for Wigeon it was 0.17 g/bird.

Discussion

These results confirm previous studies on the foraging habits of waterfowl (Baldwin and Lovvorn, 1994, 1995, 1996.) that indicate that Wigeon have the highest consumption of Japanese eelgrass of the common duck species. They suggest Japanese eelgrass could be an important food in their diet in Boundary Bay, B.C. Our results for Willapa Bay, based on dry weight analysis of stomach vegetation and the small percentage of birds with > 4 Japanese eelgrass leaves, suggests that foraging value of Japanese eelgrass across all species of duck in Willapa Bay is not as critical as suggested by Baldwin and Lovvorn. In addition, the amount of Japanese eelgrass available for forage rapidly declines (75% decrease in dry weight between 10/8 and 11/15) at the

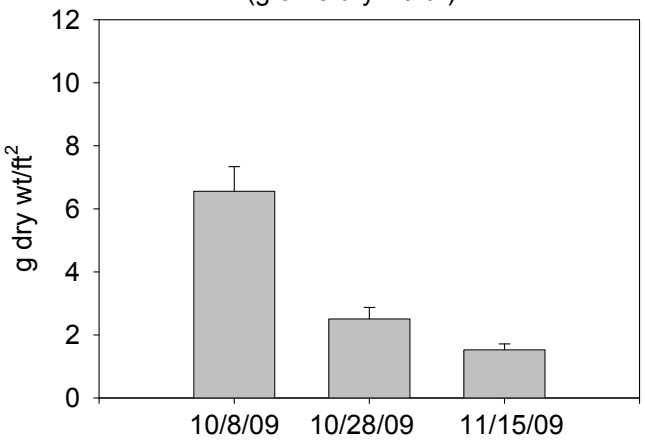
onset of fall migration (**Figure 1**). Because of its extensive spread throughout the bay (>20,000 ac) ample Japanese eelgrass will be available for waterfowl foraging , even when control of Japanese eelgrass occurs on the commercial shellfish grounds where it is a significant production pest (~2,000 to 3,000 ac). A more detailed foraging budget would be required to make additional inferences.

Table 1. Assessment of Esophagus and Proventriculus Contents of Duck Samples Collected During the Fall/Winter Hunting Season in 2009 and 2010.

Species	# bird dis-sected	year		Esophagus + proventriculus content									
				% with vege-tation	% with *Z. jap-onica*	% with *Z. japonica* leaves			% with Z. jap-onica seeds	% empty	dry wt of vegetation (g)/ bird *		
		09	10			> 1 lf.	> 2 lvs.	> 4 lvs.			N	mean	std. err.
Mallard	18	15	3	72	44	22	17	0	22	39	3	0.051	0.034
Pintail	66	33	33	71	15	6	2	0	8	38	23	0.091	0.043
Teal	14	13	1	93	43	0	0	0	14	7			
Wigeon	20	10	10	100	85	80	20	5	15	0	9	0.175	0.042
total	118	71	47	79	35	20	8	1	12	28			

*vegetation dry only collected in 2010. Mean is only from birds containing vegetation in their esophagus + proventriculus.
There was no trend for a difference in contents between different locations, other than Mallards shot on the wildlife refuge had no Z. japonica.

Figure 1. Decline in *Z. japonica* density during the fall



Samples collected Leadbetter, Oysterville and Nahcotta in 2009
n= 20, 19 and 32 for 10/8, 10/28 and 11/15 respectively.

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