PORTLAND HARBOR RI

APPENDIX G
BASELINE ECOLOGICAL RISK ASSESSMENT
ATTACHMENT 15
EVALUATION OF LAMPREY SENSITIVITY TO SEDIMENT CONTAMINANTS

DRAFT

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Prepared for
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# TABLE OF CONTENTS

1.0 INTRODUCTION ............................................................................................................. 1

2.0 LAMPRICIDE STUDIES FROM THE GREAT LAKES ................................................ 2

3.0 LITERATURE-REPORTED STUDIES OF ACUTE TOXICITY TO LAMPREY........ 4

4.0 INTERPRETATION OF ROUND 3 LAMPREY TOXICITY TESTING
   RESULTS .......................................................................................................................... 5
   4.1 Pentachlorophenol ....................................................................................................... 6
   4.2 Copper ......................................................................................................................... 7
   4.3 Diazinon ...................................................................................................................... 8
   4.4 Aniline ......................................................................................................................... 9
   4.5 Naphthalene ............................................................................................................... 10
   4.6 Lindane ....................................................................................................................... 11
   4.7 Summary .................................................................................................................... 12

5.0 CONCLUSIONS ............................................................................................................. 13

6.0 REFERENCES ................................................................................................................ 14
LIST OF FIGURES

Figure 4-1. Species Sensitivity Distribution for Pentachlorophenol .............................................6
Figure 4-2. Species Sensitivity Distribution for Copper .................................................................8
Figure 4-3. Species Sensitivity Distribution for Diazinon ............................................................9
Figure 4-4. Species Sensitivity Distribution for Aniline ...............................................................10
Figure 4-5. Species Sensitivity Distribution for Naphthalene......................................................11
LIST OF TABLES

Table 3-1. Comparison of Methylmercury LC50s for Sea Lamprey Ammocoetes with Those of Juveniles of Other Fish Species ............................................................... 4
Table 4-1. LC50, NOAEL, and LOAEL for Pentachlorophenol .................................................. 6
Table 4-2. LC50, NOAEL, and LOAEL for Copper ........................................................................ 7
Table 4-3. LC50, NOAEL, and LOAEL for Diazinon ................................................................. 8
Table 4-4. LC50, NOAEL, and LOAEL for Aniline ..................................................................... 9
Table 4-5. LC50, NOAEL, and LOAEL for Naphthalene ............................................................ 11
Table 4-6. LC50, NOAEL, and LOAEL for Lindane ................................................................. 12
1.0 INTRODUCTION

Pacific lamprey ammocoetes were selected as the detritivorous fish receptor for the baseline ecological risk assessment (BERA). The risk assessment for lamprey relies on the evaluation of two lines of evidence (LOEs):

- Comparison of water chemical concentrations to state water quality standards (WQS), national ambient water quality criteria (AWQC), or other toxicity reference values (TRVs) for aquatic organisms

- Comparison of field-collected lamprey ammocoete and macrophthalmia tissue chemical data to tissue-residue TRVs

Because lamprey are unique fish in terms of their life history (burrowing filter feeders) and are distantly related to other fish, there are uncertainties that are unique to assessing and characterizing risks to lamprey ammocoetes in Portland Harbor.

The following attachment presents a review of the toxicological literature as it relates to the susceptibility of lamprey to toxicants and a summary of the toxicological studies carried out as part of the BERA. This review and summary are presented to evaluate: 1) whether the use of non-lamprey fish thresholds in water is appropriate for evaluating risks to lamprey based on relative sensitivities, 2) whether the use of fish tissue-residue TRVs based on non-lamprey species are appropriate for evaluating risks to lamprey (comparing bioaccumulation by lamprey and other fish species), and 3) uncertainties in extrapolating acute toxicity data to chronic toxicity.

The remainder of this attachment is organized as follows:

- Section 2.0 presents a brief review of the scientific literature on lampricides.
- Section 3.0 summarizes studies investigating acute toxicity of chemicals to lamprey and compares lamprey sensitivity with that of other fish.
- Section 4.0 compares the relative sensitivity of lamprey and other fish based on the lamprey LC50 (concentration that is lethal to 50% of an exposed population) toxicity studies carried out as part of the BERA.
- Section 5.0 presents the conclusions of this lamprey toxicity evaluation.
2.0 LAMPRICIDE STUDIES FROM THE GREAT LAKES

Sea lamprey (*Petromyzon marinus*) is an exotic pest species in the Great Lakes that causes significant damage to commercial fisheries. As a result, much research has gone into the control of their populations. From 1953 to 1980, approximately 10,000 known toxicants, predominantly organic chemicals, were tested as possible controls for lamprey (Howell et al. 1980). All toxicant efforts focused on the ammocoete life stage to prevent the development of spawning adults. This effort has resulted in two classes of compounds that contain chemicals that are selectively toxic to lamprey:

- 15 halogenated mononitrophenols (e.g., 3-trifluormethyl-4-nitrophenol [TFM], the major toxicant used in sea lamprey control)
- 56 substituted nitrosalicylanilides (e.g., 5,2'-dichloro-4'-nitrosalicylanilide [niclosamide], which is used to increase the toxicity of TFM to lamprey or is applied alone).

Several studies demonstrated that lampreys are more sensitive to lampricides than other species of fish such as rainbow trout and walleye (Applegate et al. 1957; Marking et al. 1970; Dawson et al. 1975; Piavis and Howell 1975; Seelye et al. 1987; Rye and King 1976; Scholefield and Seelye 1992; Scholefield et al. 1995; Howell et al. 1964; Dawson et al. 1977). However, no other family of compounds that resulted in greater toxicity to lamprey than to other tested fish species was identified (Applegate et al. 1966; Applegate et al. 1957; Howell et al. 1980). Even within the group of chemicals selectively toxic to lamprey, the differential sensitivity between lamprey and rainbow trout is fairly small. Of 31 substituted mononitrophenols tested, 15 were selectively toxic to lamprey with ratios of rainbow trout/lamprey LC100s ranging from 1.7 for the least selective to 4.5 for the most selective lampricide, TFM (Applegate et al. 1966). In contrast, TFM is reported to have similar toxicity to lamprey and some invertebrate species (Bills and Johnson 1992; Scholefield et al. 1995; Rye and King 1976). The toxicity of TFM and niclosamide among three genera of lamprey (*Ichthyomyzon*, *Lampetra*, and *Petromyzon*) was similar (i.e., within a factor of 3) (Scholefield and Seelye 1992 and citations therein). In addition, TFM is selective for lamprey only within a narrow range of pH. Bills and Johnson (1992) reported that at a slightly basic pH of 8.4, TFM caused 100% mortality in lamprey and 0% in all other fish species tested at all treatment concentrations (2.3 to 8 mg/L). However, at a neutral pH of 7.2, mortality rates were similar among all species. At a pH of 9.2, TFM was selective of lamprey, but 100% mortality was not reached at any but the highest concentration tested.

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1 None of the members of these groups are chemicals of interest or chemicals of potential concern in Portland Harbor.

2 pH in Willamette River samples collected as part of Rounds 2a and 3 ranged from 6.3 to 8.7, with an average of 7.4 ±0.1.
Based on its chemical structure, TFM’s mechanism of action is likely through the inhibition of enzymes responsible for oxidative phosphorylation, also known as oxidative phosphorylation uncoupling (Niblett and Ballantyne 1976). When exposed to other chemicals that work through this same toxicological action, lamprey appear to have sensitivity similar to that of other fish species. The model oxidative phosphorylation uncoupler is 2, 4-dinitrophenol. Applegate (1957, 1966) included 2,4-dinitrophenol in the comparison test between lamprey, trout, and bass. Lamprey and bass showed similar sensitivity, whereas trout were more sensitive. Based on Pacific lamprey pentachlorophenol LC50 toxicity data from Round 3 water-only toxicity studies (Windward and Integral 2008) and LC50 data for other fish from the ambient water quality criteria (EPA 1986), lamprey ammocoetes have LC50s similar to those for trout and salmonid species and are less sensitive to pentachlorophenol than approximately 15% of the species tested (see Section 4.0). Lech and Statham (1975) demonstrated that lamprey have a lower rate of biotransformation of TFM compared to rainbow trout, and they hypothesized that this was one reason for lamprey’s greater sensitivity to TFM compared to trout. When biotransformation of TFM was blocked in both species, rainbow trout became more sensitive to TFM, whereas lamprey sensitivity did not change (Lech and Statham 1975).

In summary, the different sensitivity to lampricides between lamprey and rainbow trout was relatively small based on ratios of rainbow trout/lamprey LC100s ranging from 1.7 to 4.5. The toxicity of lampricides among three genera of lamprey was similar (i.e., within a factor of 3). Lampricide is selective for lamprey only within a narrow range of pH; outside this range (as is typical in the Willamette River in Portland Harbor), mortality rates were similar among all species. The mode of action for lampricides is the uncoupling of oxidative phosphorylation. When exposed to other chemicals with the same mode of action, lamprey and other fish appear to have similar sensitivity.

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3 Oxydative phosphorylation releases energy from the oxidation of nutrients to form adenosine triphosphate, which supplies the critical energy for metabolism.

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3.0 LITERATURE-REPORTED STUDIES OF ACUTE TOXICITY TO LAMPREY

Literature-reported acute toxicity studies with lamprey were identified for methylmercury and the organochlorine insecticide, kepone. Sea lamprey and other fish appear to have similar sensitivity to methylmercury (Table 3-1).

Table 3-1. Comparison of Methylmercury LC50s for Sea Lamprey Ammocoetes with Those of Juveniles of Other Fish Species

<table>
<thead>
<tr>
<th>Species Common Name</th>
<th>Species Name</th>
<th>Methylmercury LC50 (µg Hg/L)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea lamprey</td>
<td>Petromyzon marinus</td>
<td>&gt; 166</td>
<td>Mallatt et al. (1986)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Oncorhynchus mykiss</td>
<td>52 to 125 ND</td>
<td>Matilda et al. (1971); Wobeser (1975)</td>
</tr>
<tr>
<td>Brook trout</td>
<td>Salvelinus fontinalis</td>
<td>ND</td>
<td>McKim et al. (1976)</td>
</tr>
<tr>
<td>Blue (3-spot) gourami</td>
<td>Trichogaster trichopterus</td>
<td>ND</td>
<td>Roales and Perlmutter (1974, as cited in Mallatt et al. 1986)</td>
</tr>
<tr>
<td>Mummichog</td>
<td>Fundulus heteroclitus</td>
<td>ND</td>
<td>Sharp and Neff (1982, as cited in Mallatt et al. 1986)</td>
</tr>
</tbody>
</table>

Source: Adapted from Mallatt et al. (1986).

Hg – mercury
LC50 – concentration that is lethal to 50% of an exposed population
ND – no data

Available acute toxicity data show that larval sea lamprey tolerate higher levels of kepone than do all other fish species (Mallatt and Barron 1988). At 12 °C, the 36-hr LC50, 96-hr LC50, and incipient lethal concentrations were 1,100, 444, and 145 µg kepone/L, respectively, while at 20 °C, the 96-hr LC50 was 414 µg kepone/L.

A study investigating lamprey survival in high-iron-content rivers in Finland, reported that lamprey egg hatchability and newly hatched larval survival was reduced by high iron and low pH conditions (Myllynen et al. 1997). This study indicates that water chemistry can influence metals toxicity thresholds, which is common for other fish species as well. These acute studies indicate that the sensitivity of lamprey to some chemicals is similar to or less than other fish species.
4.0 INTERPRETATION OF ROUND 3 LAMPREY TOXICITY TESTING RESULTS

A sensitivity study of lamprey ammocoetes (*Lampetra* sp.) was conducted in response to a request from EPA (2006) to determine whether aquatic toxicological water thresholds (i.e., water TRVs) used in the Portland Harbor BERA are protective of lamprey survival and growth at the organism level. More specifically, the study was performed to meet the following two general objectives:

- Determine whether existing fish TRVs are sufficiently protective of lamprey survival and growth as determined by laboratory testing with representative chemicals
- Evaluate the sensitivity of Pacific lamprey to chemical toxicity with that of other fish by comparing the lamprey data with published toxicity data for the most sensitive surrogate species

The study design included the toxicity testing of six chemicals (i.e., pentachlorophenol, copper, aniline, diazinon, naphthalene, and lindane) selected to represent a range of toxicity modes of action (e.g., narcosis and interference with metabolic pathways, osmoregulation, and neurotransmitter activity) to assess if TRVs based on standard test species would be protective of lamprey ammocoetes. The study confirmed that across the tested modes of action, rainbow trout or other salmonids were at least as sensitive as lamprey ammocoetes. The objectives, methods, procedures, and results for the acute toxicity testing were presented in two data reports (Windward 2007; Windward and Integral 2008).

This section presents an interpretation of the acute toxicity testing, including a description of modes of action and a comparison of the sensitivity of lamprey ammocoetes relative to that of other aquatic species. The relative sensitivity of lamprey ammocoetes was evaluated using a species sensitivity distribution (SSD), which displays available toxicity data as a plot of the LC50 for each species on the x-axis and the cumulative probability (estimated fraction of species with LC50s at or below the corresponding LC50 value) on the y-axis. The measured lamprey LC50 concentrations were compared to LC50s for all other aquatic species for which toxicological data were available. The SSDs were made using @Risk 5.0 for Microsoft Excel®. The best distribution for each chemical was selected using three goodness-of-fit tests (i.e., Chi-Square, Kolmogorov-Smirnoff, and Anderson-Darling), fitness ranking provided by the statistical program, and professional judgment. The SSDs are based on available toxicity data for aquatic species, including fish, invertebrates, and amphibians.
4.1 PENTACHLOROPHENOL

The LC50, no-observed-adverse-effect level (NOAEL), and lowest-observed-adverse-effect level (LOAEL) for pentachlorophenol calculated from the ammocoete bioassay results conducted for the BERA are presented in Table 4-1. Based on this study and data from the ambient water quality criteria development document (EPA 1986), lamprey ammocoetes have LC50s similar to those for trout and salmonid species and fall at approximately the 15th percentile of the pentachlorophenol SSD (Figure 4-1) using the Pearson6 distribution. Pentachlorophenol is an oxidative phosphorylation uncoupler that interferes with an organism’s ability to synthesize adenosine triphosphate (ATP), which supplies the critical energy for metabolism (McKim et al. 1987). This is also the identified mode of action for trifluoromethyl-4-nitrophenol, a chemical commonly used to control for sea lamprey (Niblett and Ballantyne 1976).

Table 4-1. LC50, NOAEL, and LOAEL for Pentachlorophenol

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 µg/L</td>
<td>28 – 34 µg/L</td>
<td>17 µg/L</td>
<td>30 µg/L</td>
</tr>
</tbody>
</table>

LC50 – concentration that is lethal to 50% of an exposed population
LOAEL – lowest-observed-adverse-effect level
NOAEL – no-observed-adverse-effect level

Figure 4-1. Species Sensitivity Distribution for Pentachlorophenol
4.2 COPPER

The LC50, NOAEL, and LOAEL for copper calculated from the ammocoete bioassay results are presented in Table 4-2. The sensitivity of lamprey ammocoetes to copper approximated the average of aquatic species tested (46th percentile of the distribution; Figure 4-2) based on an LC50 of 46 µg/L, data used to develop the ambient water quality criteria (EPA 2007b), and the Pearson6 distribution. Numerous fish species, including northern squawfish, rainbow trout and other trout species, darter and several salmon species are all more sensitive to copper than are lamprey ammocoetes. Copper interferes with osmoregulation in fish by inhibiting the enzyme (Na, K ATPase) regulating the uptake of sodium (Na+) and chloride (Cl-) ions in gill tissue. The decrease in plasma electrolytes causes water to move from the blood into the tissues, and the resulting hemoconcentration causes increased arterial blood pressure and finally heart failure (Heath 1995). Another mechanism of osmoregulation interference has been reported for lamprey. Exposure to copper sulfate (CuSO₄) at a concentration of 100 µM caused anion-dependent intracellular acidification and an increase in Cl⁻ influx in lamprey erythrocytes (Bogdanova et al. 1999). In addition, the copper treatment caused an increase in Na⁺ fluxes in both directions and a net Na⁺ uptake. The resulting cellular swelling induced a regulatory volume decrease response involving potassium (K⁺) chloride extrusion via K⁺ and Cl⁻ volume-sensitive channels.

Table 4-2. LC50, NOAEL, and LOAEL for Copper

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>46 µg/L</td>
<td>42 – 51 µg/L</td>
<td>23 µg/L</td>
<td>43 µg/L</td>
</tr>
</tbody>
</table>

LC50 – concentration that is lethal to 50% of an exposed population
NOAEL – no-observed-adverse-effect level
LOAEL – lowest-observed-adverse-effect level
4.3 DIAZINON

The LC50, NOAEL, and LOAEL for diazinon from the ammocoete bioassays are presented in Table 4-3. Lamprey ammocoetes were relatively insensitive to diazinon when compared with other aquatic species, as indicated by the LC50 of 8.9 mg/L, which falls approximately at the 72nd percentile of the SSD (Figure 4-3) based on data from the ambient water quality criteria development document (EPA 2005) and the BetaGeneral distribution. Zebrafish, fathead minnow, flagfish, guppy, bluegill, and several species of trout are all more sensitive to diazinon than are lamprey ammocoetes. Diazinon inhibits acetylcholinesterase, an enzyme required for the metabolism of the neurotransmitter acetylcholine. This inhibition increases both the activity level and duration of the neurotransmitter (Denton et al. 2003), which can result in uncontrollable movement (e.g., twitching) and ultimately paralysis.

Table 4-3. LC50, NOAEL, and LOAEL for Diazinon

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.9 mg/L</td>
<td>8.5 – 9.4 mg/L</td>
<td>6.3 mg/L</td>
<td>12.5 mg/L</td>
</tr>
</tbody>
</table>

LC50 – concentration that is lethal to 50% of an exposed population
LOAEL – lowest-observed-adverse-effect level
NOAEL – no-observed-adverse-effect level
4.4 ANILINE

The LC50, NOAEL, and LOAEL for aniline calculated from the ammocoete bioassay results are presented in Table 4-4. Lamprey ammocoetes were relatively insensitive to aniline when compared with other aquatic species, as indicated by the LC50 of 433 mg/L, which falls at approximately the 90th percentile of the SSD (Figure 4-4) based on data from the ambient water quality criteria development document (EPA 1993) and the exponential distribution. Goldfish, fathead minnow, white sucker, bluegill, and rainbow trout are all more sensitive to aniline than are lamprey ammocoetes. The aniline toxic mode of action has been identified as polar narcosis (Bearden and Schultz 1997). In general, narcosis results in paralysis of an organism and may cause death if respiration is suppressed or the organism becomes vulnerable to predation. A large number of industrial organic chemicals exert their toxic effects through this mode of action.

Table 4-4. LC50, NOAEL, and LOAEL for Aniline

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>433 mg/L</td>
<td>381 – 486 mg/L</td>
<td>318 mg/L</td>
<td>634 mg/L</td>
</tr>
</tbody>
</table>

LC50 – concentration that is lethal to 50% of an exposed population
NOAEL – no-observed-adverse-effect level
LOAEL – lowest-observed-adverse-effect level
4.5 NAPHTHALENE

The LC50, NOAEL, and LOAEL for naphthalene based on the ammocoete bioassays are presented in Table 4-5. An LC50 could not be calculated for naphthalene because of limited mortality in the lamprey ammocoete toxicity test. However, an LC50 of 10.18 mg/L was estimated based on 50% mortality in the highest concentration tested. Lamprey ammocoetes were relatively insensitive to naphthalene when compared with other aquatic species as indicated by the fact that the estimated LC50 fell at approximately the 85th percentile of the SSD (Figure 4-5) based on data from the ambient water quality criteria documents (EPA 1980b, 2008) and the Pearson5 distribution. Steelhead trout, rainbow trout, bluegill, and fathead minnow are all more sensitive to naphthalene than are lamprey ammocoetes. The naphthalene toxic mode of action has been identified as narcosis (Broderius et al. 2005).
Table 4-5. LC50, NOAEL, and LOAEL for Naphthalene

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.18 mg/L$^a$</td>
<td>NA</td>
<td>5.3 mg/L</td>
<td>10.18 mg/L</td>
</tr>
</tbody>
</table>

$^a$ Estimated concentration based on 50% mortality in the highest test concentration.

LC50 – concentration that is lethal to 50% of an exposed population
LOAEL – lowest-observed-adverse-effect level
NA – not available
NOAEL – no-observed-adverse-effect level

Figure 4-5. Species Sensitivity Distribution for Naphthalene

4.6 LINDANE

The LC50, NOAEL, and LOAEL for lindane are presented in Table 4-6. An LC50 could not be calculated for lindane because there was only 12.5% mortality in the highest concentration that the laboratory was able to get into solution. The highest lindane concentration (2.68 ±0.73 mg/L) was near saturation based on information available in the literature. Two studies reported a water solubility of 2.15 mg/L at 15 ºC (Biggar et al. 1968; Ivanov 1956), and one study reported a solubility of 8.9 mg/L at 19 ºC (Biggar et al. 1968). The ambient water quality criteria for lindane included a solubility range of 10 to 32 mg/L (EPA 1980a). However, the temperature at which this solubility was measured was not included. Lamprey ammocoetes are insensitive to lindane based on a comparison of the low

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mortality rate at water concentrations approaching saturation with the LC50s of other fish, which range from 0.001 to 0.24 mg/L. Numerous fish species, including trout, salmon, carp, largemouth bass, sunfish, catfish, and perch are more sensitive to lindane than are lamprey ammocoetes (EPA 1980a, 2007a). Lindane reduces the activity of the neurotransmitter serotonin, which may have important physiological consequences because the serotoninergic system in fish is involved in processes such as neuroendocrine regulation, growth, and reproduction (Aldegunde et al. 1999).

Table 4-6. LC50, NOAEL, and LOAEL for Lindane

<table>
<thead>
<tr>
<th>LC50</th>
<th>95% Confidence Limit</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2.68 mg/L</td>
<td>NA</td>
<td>2.68 mg/L</td>
<td>&gt; 2.68 mg/L</td>
</tr>
</tbody>
</table>

LC50 – concentration that is lethal to 50% of an exposed population
LOAEL – lowest-observed-adverse-effect level
NA – not available
NOAEL – no-observed-adverse-effect level

4.7 SUMMARY

Toxicity data established in Round 3 water-only toxicity tests indicated that lamprey have similar or lower sensitivity to other fish.

The study confirmed that across the tested modes of action, rainbow trout or other salmonids were at least as sensitive as lamprey ammocoetes. Of the six test chemicals, lamprey ammocoetes were most sensitive to pentachlorophenol, which is representative of the oxidative phosphorylation uncoupling mode of action. However, even in the case of pentachlorophenol, the ammocoetes were not more sensitive than rainbow trout. The sensitivity of lamprey ammocoetes to copper approximated the average for other aquatic species. For the other four chemicals (i.e., aniline, diazinon, naphthalene, and lindane), the sensitivity of lamprey ammocoetes fell within the upper percentiles of their respective SSDs, indicating that lamprey ammocoetes were less sensitive than were most aquatic species tested.

Because lamprey were found to be equally or less sensitive than rainbow trout and other salmonids for all of the six chemicals tested, the aquatic toxicological thresholds (i.e., water TRVs) used in the BERA are sufficiently conservative for this measurement endpoint.
5.0 CONCLUSIONS

Lamprey are distantly related to other fish and have some unique physiology. Laboratory studies indicate that lamprey accumulate mercury more readily than do other fish. With the exception of high mercury bioaccumulation, the relevance of their physiology to toxicity is uncertain. Toxicity studies indicate that lamprey are insensitive to mercury.

Because sea lamprey are a pest species in the Great Lakes, they have been the subject of extensive studies comparing their sensitivity to toxic chemicals to that of other aquatic organisms. With the exception of lampricides, lamprey tend to be less or equally sensitive to chemical toxicity as other fish. Lamprey’s relative insensitivity is confirmed by the results of lamprey water exposure LC50 studies conducted for the BERA.

Lamprey LC50 studies were based on aqueous exposure, whereas, in Portland Harbor, fish are exposed through direct sediment contact, water, and diet. These differences could result in different relative sensitivities among aquatic organisms based on tissue-residue and water toxicity data. In general toxicity from direct sediment exposure is the result of exposure to interstitial water, thus the relative sensitivity of chemicals from water and sediment exposures should be similar. In small animals, uptake across the dermis can be an important route of exposure; like gill uptake, chemicals pass directly into the bloodstream so gill and dermal uptake should result in similar internal fates and thus similar toxic effects. In short-term water exposures, tissue residues tend to be higher in gills than in other tissues and in short-term dietary exposures, tissue residues tend to be higher in the gut. In longer-term exposures, tissue residues are generally distributed following equilibrium partitioning. Few studies are available to directly compare residue effects thresholds arising from dietary and water exposures. Uncertainty associated with exposure route is similar for lamprey and other fish. In addition, lamprey LC50s are based on acute 96-hour exposures; whereas, in Portland Harbor, lamprey may be exposed for up to 6 years, based on their life history. Uncertainty associated with use of acute data to determine lamprey’s relative sensitivity is similar to that for other fish.
6.0 REFERENCES


EPA. 2007a. EPA e-mail dated December 27, 2007 (B. Shephard to J. Toll, Windward Environmental) regarding lamprey toxicity relative to other species with attached Excel file. US Environmental Protection Agency Region 10, Seattle, WA.


EPA. 2008. EPA e-mail dated May 30, 2008 (B. Shephard to J. Toll, Windward Environmental) regarding unpublished naphthalene LC50 data with attached Excel file. US Environmental Protection Agency Region 10, Seattle, WA.


