Northwest Environmental Advocates

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Washington State Habitat Office
510 Desmond Drive SE, Suite 103
Lacey, WA 98503
January 10, 2008

Mr. Mike Gearheard
Director, Office of Water and Watersheds
U.S. Environmental Protection Agency, Region 10
(OWW130)
1200 Sixth Avenue
Seattle, Washington 98101

Dear Mr. Gearheard:

The State of Washington Department of Ecology (Ecology) has recently issued a Public Notice Draft National Pollution Discharge Elimination System (NPDES) Industrial Stormwater General Permit for public review and comment. With the CWA authority delegated from the EPA, Ecology proposes to reissue the Industrial Stormwater General Permit to over 1,150 industrial facilities in Washington State, revoking and replacing the current permit.

We support Ecology's objectives in permitting a large number of industrial facilities, which will reduce the discharge of contaminated stormwater from industrial activities into receiving waters, and help protect fish and wildlife resources including threatened and endangered salmon. We are pleased that the Draft Industrial Stormwater General Permit increases the level of protection for listed salmon by reducing the total copper threshold from 63.9 µg/L to 20 µg/L. We expect that the change in the copper threshold would minimize copper loadings in some waterbodies within the State, over those authorized by the current permit. However, we must also note that we do not expect the copper threshold levels within the permit will completely eliminate adverse effects to salmon, including species listed under the Endangered Species Act. In particular, the scientific information available to us suggests that behavioral and physiological effects of dissolved copper to listed salmon may occur at values ranging from 0.18 to 2.1 µg/L in freshwater (Hecht et. al, 2007).

We are also concerned that, given the frequency and timing of monitoring in the draft permit, the likelihood of missing storm events where discharges exceed a benchmark or threshold for copper or other pollutants is high. Further, Ecology's reliance on the central tendency of the data suggests that some facilities may be able to exceed permit conditions, including effluent standards, and not be required to address these exceedances. Others may be able to exceed effluent standards for months or years before taking corrective actions. In the interim, some runs of listed salmon and their critical habitat could be repeatedly exposed to sufficiently high copper, zinc, and other pollutant

levels that may cause serious behavioral and physiological consequences before corrective action is taken to minimize loadings in receiving waters.

According to the processes outlined in the Memorandum of Agreement (hereafter "MOA") (May 22, 2001, 66FR 11202-11217, Section IX.A., 3.-6.), between the Environmental Protection Agency (EPA) and the National Marine Fisheries Service (NMFS) regarding enhanced coordination under the Clean Water Act (CWA) and Endangered Species Act (ESA), we have met with Ecology to discuss our identified concerns with the permit. We are sending these comments to you because of EPA's acknowledged oversight role in the issuance of this permit under Section 402(d) of the Clean Water Act (CWA), and acknowledged responsibility to comply with Section 7(a)(2) of the Endangered Species Act (ESA). As per the MOA, this letter serves written notice of our phone contact to you on January 4, 2008, relaying our concern that the stormwater discharges authorized under this permit --- even though they are a major improvement over the current levels --- are still at levels likely to have more than minor detrimental effects to ESA listed salmon and critical habitat.

The geographic area covered by the permit overlaps the range of 15 federally-listed threatened or endangered salmon, as well as designated critical habitat for 13 of these populations. The permit area overlaps areas addressed by the Puget Sound Shared Strategy Recovery Plans, Lower Columbia River Fish Recovery Board, the Upper and Mid-Columbia Fish Recovery Boards, the Governor's Salmon Plan, and the Puget Sound Partnership. Most of these plans have identified stormwater runoff as a significant factor in reaching salmon recovery. In addition, the Puget Sound Partnership has developed recommendations for addressing stormwater effects with the goal of achieving a healthy Puget Sound by the year 2020. Also, a recent report supported by your agency, identified stormwater runoff as the greatest contributor of the worst pollutants in Puget Sound (Hart Crowser, Inc. et al. 2007). Therefore, we encourage Ecology and EPA to continue to reduce pollutant levels, including dissolved copper levels, to State waters.

We look forward to continued coordination with EPA and Ecology on NPDES permits, as well as completing our ESA consultations on Water Quality Standards as they are revised in Washington State and at the National level. Please note that our National Office is currently engaged in formal consultation with EPA on the national Mulitsector General Permit, which covers industrial stormwater discharges in non-delegated states and Federal and Tribal lands in delegated states, including Washington. Through our continued effort with EPA at the national level on the Multisector general permit, NOAA expects to present further information and engage in further discussions that should help inform both national water quality standards and state water quality standards. We expect that consultation to consider not only copper but also other heavy metals of concern. In the course of these discussions, we may suggest additional ways to minimize adverse effects of stormwater on NMFS' trust resources. While we are encouraged by

the progress that Ecology has made on their proposed industrial stormwater permit, our comments on the this Draft Stormwater Industrial General Permit should not be understood to limit or otherwise prejudice our recommendations in the future on national or regional actions.

Thank you for the opportunity to provide these comments under the process identified in the MOA. Please call me at (360) 753-6054 if you would like to discuss this issue further.

Sincerely,

Steven W. Landino
Washington State Director
for Habitat Conservation

Attachments: (2)

cc: Dave Peeler, Ecology
Ken Berg, USFWS

Attachment A: NOAA Fisheries Review of the NPDES Industrial Stormwater General Permit

Waters affected by the industrial permit are important to the ecology of salmonids listed as threatened or endangered pursuant to the Endangered Species Act of 1973, as amended (ESA). Based on the body of scientific evidence available, these discharges are likely to produce water quality conditions that have behavioral and physiological consequences for salmon that may reduce the viability of populations exposed to those conditions. The purpose of this attachment is to describe and support this conclusion with available scientific and commercial information. Our review analyzes the primary effects of the Industrial Permit and the stormwater discharges it authorizes on listed salmonids, concentrating on pollutant levels, monitoring and corrective actions.

Pollutant levels

In this section, we focus on copper and zinc as examples of pollutant benchmark levels proposed in the permit that we expect will have more than minor detrimental effects on salmonids. While we expect that levels of other pollutants such as TSS, lead, nitrate, phosphorus, and others covered in the permit also have adverse effects, we focused our review on the effects of copper and zinc to illustrate our concerns.

Salmon experience adverse effects at 2 µg/L dissolved Cu (Hecht et al 2007) and 5.6 µg/L dissolved Zn (Sprague 1968). For copper, these effects include interference with fish sensory systems and important behaviors that underlie predator avoidance, juvenile growth and migratory success. For zinc, these effects include altered behavior, blood and serum chemistry, impaired reproduction, and reduced growth. These effects occur at pollutant levels that are 10 and 20 times lower than the benchmark levels of 20 µg/L total Cu and 115 µg/L total Zn specified in the proposed Industrial permit. About one quarter of the permittees will be discharging to 303(d) listed waters and will have to meet a stricter benchmark of 7 µg/L total Cu and 77.5 µg/L total Zn. These benchmarks are still approximately 3.5 and 14 times higher than the level at which adverse effects occur to salmon. An example of adverse effects at an approximate benchmark level has been documented using a short-term exposure to 20 µg/L dissolved Cu (approximate benchmark level), which reduced the olfactory response of salmon by 82 percent (McIntyre et al, in press). Significant impairment of sensory functions may occur following 10 minutes of low level exposure and continue for hours to weeks depending on concentration and duration.

According to a report prepared for Ecology (Envirovision et al 2006), benchmarks were selected using a Simple Percentile method and individual facility median pollutant values equivalent to the 50th percentile of the available data. This level equates to the level of pollutant discharge that half of the permittees have managed to attain. In other words, it appears the resulting benchmarks of 20 µg /L total Cu and 115 µg/L total Zn were chosen based on treatment technologies, not State water quality standards. The state water quality acute and chronic criteria are more stringent for Cu (13 µg/L (chronic) and 9 µg/L (acute) dissolved) compared to 20 µg/L total Cu (based on 100mg.l hardness). However,

the same Ecology report (Envirovision et al 2006) states that "...permit targets generally contain effluent limits based on State water quality standards or treatment technologies and the most stringent of these two limits, must be chosen to establish the permit limit for each parameter of concern. We believe the implementation of this lower target level is an improvement over past target levels and as a result, may lead to corrective actions early than had occurred under the current permit. Nevertheless, we are concerned that this lower target level would still result stormwater discharges of dissolved copper and other pollutants that have more than a minor detrimental effect on listed salmon species and their designated critical habitat.

Monitoring

Ecology has had an Industrial permit in effect in Washington State since 1992. Between 1992 and 2000, monitoring was conducted through on-site field visits by Ecology staff. After 2002, permittees were required to start collecting their own monitoring information and report exceedances of permit limits to Ecology. In the proposed permit, sampling times have changed to catch rain events and sampling frequency was increased from 4 to 5 times/year (on the west side). Sampling in the proposed permit would also include sampling for total Cu and Zn, which is not included in the current permit.

With the wide-ranging variation in storm events, facility sites, and pollutants encountered, we believe sampling 5 times a year is not adequate to provide data that accurately portray the pollutant concentrations generated from these sites. In addition, sampling in total Cu and total Zn instead of sampling for the dissolved fraction of these metals, does not give accurate information about the bioavailable metals fraction that can result in direct and short term toxicity to salmonid sensory systems, sensitive salmonid prey (aquatic insects), and primary producers. We are unsure why Ecology requires sampling for total instead dissolved metals as recent policy from USEPA's Office of Water mandates the use of dissolved metal to set and measure compliance with water quality standards (Sanalone et al 1997).

To develop the sampling requirements and pollutant benchmarks and thresholds, many assumptions were made about critical variables that affect the toxicity of stormwater discharges (Envirovision 2006), e.g., dilution levels, water hardness, dissolved versus total fractions of metals, etc. Requiring permittees to monitor these parameters would provide much more accurate information on which to base permit target levels, e.g., stormwater discharge (in cubic feet per second (cfs)), streamflow cfs, water hardness, total suspended solids, background metals levels, etc. So, while the change in sampling times, frequencies, and inclusion of Cu and Zn sampling are an improvement over the existing permit, we believe these changes in monitoring actions are not sufficent to avoid adverse effects to listed salmon.

Corrective Actions

According to the proposed permit, if benchmarks or thresholds are exceeded the permittee performs corrective actions. If a threshold is exceeded, the permittee notifies Ecology and Ecology visits the site In the pollutant section above, we have already discussed that pollutant benchmark and threshold levels, especially for Cu and Zn, are too high to avoid effects to salmon. In addition, once a permittee has collected their 5 samples for the year, they calculate the median of all 5 samples, and if the median is not above the benchmark, they are not required to take any corrective actions. This is in spite of the possibility that the benchmark could have been exceeded once or more in samples that year, with the prospect that the discharge from the facility could be continuing to exceed the benchmark over some extended period of time during the year. In addition, once the benchmark median is exceeded, permittees are only required to identify the need for change and any remedial actions. Actual changes to source and operation BMPs are not required until the permittee has exceeded the benchmark for a second year. To adequately protect salmon, the reporting and corrective actions should be more timely, e.g., immediate action on any one reading that exceeds the benchmark. The requirement for immediate action would match that required for non-compliance with the permit (discharge of pollutants in a significant amount) which also requires the submittal of a detailed report to Ecology in 30 days or less.

The use of means, medians, and outliers in analyzing the monitoring data has meant that the permit relies heavily of the use of the central tendency of the data. While this may work well for getting permittees to do a better job of minimizing their pollutant discharge, it does not work well minimizing pollutant effects on salmon. Finally, the values upon which these decisions are made, and the timing of implementing the actions are not likely to avoid more than minor detrimental effects to listed salmon.

References:

Envirovision and Herrera Enviornmental Consultants. 2006. Evaluation of Washington's Industrial Stormwater General Permit. Prepared for Washington Dept. of Ecology Contract no. C0600124.

Hecht, S.A., D.H. Baldwin, C.A. Mebane, T. Hawkes, S.J. Gross, and N.L. Scholz. 2007. An Overview of sensory effects on juvenile salmonids exposed to dissolved copper: Applying a benchmark concentration approach to evaluate sublethal neuro behavioral toxicity. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-83, 39p.

McIntyre, J.K., D.H. Baldwin, J.P. Meador, N.L. Scholz. (in press). Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. Environmental Science and Technology.

Sansalone, J.J. and S.G. Buchberger. 1997. Partitioning and first flush of metals in urban roadway storm water. Journal of Environmental Engineering. Feb 1997.

Sprague, J. B. 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Research Pergamon Press. Vol 2, pp. 367-372.

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Washington State Habitat Office
510 Desmond Drive SE, Suite 103
Lacey, WA 98503

July 15, 2009

Mr. Mike Gearheard
Director, Office of Water and Watersheds
U.S. Environmental Protection Agency, Region 10
(OWW130)
1200 Sixth Avenue
Seattle, Washington 98101

Dear Mr. Gearheard:

The State of Washington Department of Ecology (Ecology) has recently issued a Public Notice Draft National Pollution Discharge Elimination System (NPDES) Industrial Stormwater General Permit for public review and comment. The National Marine Fisheries Service (NMFS) offers the following brief comments on the proposed permit reissuance pursuant to our role as providers of biological and technical assistance under the Endangered Species Act of 1973 (16 U.S.C. 1531 *et seq.*), as amended (ESA) and the Fish and Wildlife Coordination Act (16 U.S.C. 661 *et seq.*). We are sending these comments to you because of EPA's acknowledged oversight role in the issuance of this permit under Section 402(d) of the Clean Water Act (CWA), and acknowledged responsibility to comply with Section 7(a)(2) of the Endangered Species Act (ESA). In addition, these comments are provided per the processes outlined in the Memorandum of Agreement between the EPA and the NMFS regarding enhanced coordination under the CWA and ESA (hereafter "MOA") (May 22, 2001, 66FR 11202-11217).

With the CWA authority delegated from the EPA, Ecology proposes to reissue the Industrial Stormwater General Permit to over 1,200 industrial facilities in Washington State, replacing the current permit. The permit uses the concept of benchmarks and action levels (levels of industrial contaminants that will require the permittee to take further actions) rather than requiring compliance with State water quality standards. In addition, the permit relies heavily a water quality risk evaluation (Herrera Environmental Consultants 2009) to justify their proposed benchmark and action levels.

The geographic area covered by the permit overlaps the range of 15 federally-listed threatened or endangered salmon, as well as designated critical habitat for 13 of these populations. The permit area overlaps areas addressed by the Puget Sound Shared Strategy Recovery Plans, Lower Columbia River Fish Recovery Board, the Upper and

Mid-Columbia Fish Recovery Boards, the Governor's Salmon Plan, and the Puget Sound Partnership. Most of these plans have identified stormwater runoff as a significant factor in reaching salmon recovery. In addition, the Puget Sound Partnership has developed recommendations for addressing stormwater effects with the goal of achieving a healthy Puget Sound by the year 2020. Also, a recent report supported by your agency, identified stormwater runoff as the greatest contributor of the worst pollutants in Puget Sound (Hart Crowser, Inc. et al. 2007).

We support Ecology's objectives in permitting this large number of industrial facilities, with the hope that the discharge of contaminated stormwater from industrial activities into receiving waters will be reduced, and fish and wildlife resources including threatened and endangered salmon will receive additional protection. However in our review of the draft permit we are not assured that protection for listed salmon will be improved. We have identified three main issues that contribute to this concern:

1) the copper and zinc benchmark levels,
2) using zinc as a surrogate for copper and limiting copper monitoring, and
3) the reliance on risk assessment calculations to protect listed species.

We have identified in the past through meetings, e-mails, and correspondence (between NMFS, EPA and Ecology) our concerns about copper and zinc levels allowed by this permit. Adverse effects of dissolved copper and zinc on listed salmon occur at very low levels (values ranging from 0.18 to 2.1 µg/L in freshwater for copper (Hecht et. al, 2007) and at 5.6 µg/L in freshwater for zinc (Sprague 1968)). Adverse effects of copper include interference with fish sensory systems and important behaviors that underlie predator avoidance, juvenile growth and migratory success. These effects occur at pollutant levels that are 6 to 77 times lower than the proposed benchmark level for total copper (14 µg/L). Similarly, adverse effects of zinc include altered behavior, blood and serum chemistry, impaired reproduction, and reduced growth. These effects occur at pollutant levels that are 35 and 45 times lower than the proposed total zinc benchmark levels (200 µg/L for Western Washington and 255 µg/L for Eastern Washington). In addition, the proposed benchmark level for zinc in this permit (200 and 255µg/L total Zn) is higher than the level proposed for the 2007 Industrial permit (115 µg/L total Zn). We do not believe these proposed benchmark levels avoid more than minor detrimental effects to listed salmon and steelhead.

Given that copper has adverse effects on listed fish at very low levels, we are surprised that Ecology has proposed in this permit to eliminate the requirement for facilities to conduct monitoring for copper when zinc benchmarks are exceeded in stormwater discharges. Instead Ecology is proposing to use total zinc as the representative metal for core sampling and apply copper sampling requirements to only 5 sectors of industrial facilities. With the proposed benchmark level for zinc set at a level that does not provide protection necessary for salmon growth and survival, and with copper being identified as a widespread pollutant in industrial facilities, we do not believe using zinc as a surrogate of copper and limiting copper monitoring to 5 sectors will adequately protect listed salmon.

The proposed permit targets for the Industrial permit are based on a water quality risk evaluation that examines the risk of exceeding acute water quality standards (Herrera Environmental Consultants 2009). For this analysis, Ecology determined that the proposed benchmarks and action levels should be considered based on a dilution factor of 5 and a 10 percent risk for exceeding the applicable water quality standard for each metal. While this may be a viable approach for setting benchmark levels across a broad range of facility types and receiving waters, it is not an approach that provides adequate protection for listed salmon. We cannot accurately assume that a dilution factor of 5 will always be provided where listed salmon are present. Nor can we accurately assume that a 10 percent risk of exceeding applicable water quality standards will not have adverse effects on listed fish, particularly when we know that current water quality standards for some pollutants (particularly copper and zinc) already exceed levels that result in adverse effects for listed salmon and steelhead. Therefore, we do not believe more than minor detrimental effects to listed salmon and steelhead will be avoided.

We thank you for the opportunity to provide these comments under the process identified in the MOA. We look forward to continued coordination with EPA and Ecology on NPDES permits, as well as completing our ESA consultations on Water Quality Standards as they are revised in Washington State, in part to meet the needs of listed salmon. Please call me at (360) 753-6054 if you would like to discuss this issue further.

Sincerely,

Steven W. Landino
Washington State Director
for Habitat Conservation

cc: Kelly Susewind, P.E., P.G. Ecology
Ken Berg, USFWS

References:

Hart Crowser, Inc. 2007. Control of Toxic Chemicals in Puget Sound. Phase 1: Initial Estimate of Loadings. Prepared for Washington State Department of Ecology, U.S. Environmental Protection Agency, and Puget Sound Partnership. Publication No. 07-10-079.

Hecht, S.A., D.H. Baldwin, C.A. Mebane, T. Hawkes, S.J. Gross, and N.L. Scholz. 2007. An Overview of sensory effects on juvenile salmonids exposed to dissolved copper: Applying a benchmark concentration approach to evaluate sublethal neuro behavioral toxicity. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-83, 39p.

Herrera Environmental Consultants. 2009. Water Quality Risk Evaluation for Proposed Benchmarks/Action Levels in the Industrial Stormwater General Permit. Analysis Report prepared for Washington State Department of Ecology. 40 pgs.

Sprague, J. B. 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Research Pergamon Press. Vol 2, pp. 367-372.

Bc: WSHO – Chron File
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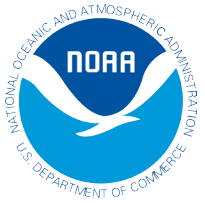
NOAA Technical Memorandum NMFS-NWFSC-135



Exposure to a Mixture of Toxic Chemicals:

Implications for the Health of Endangered Southern Resident Killer Whales

doi:10.7289/V5/TM-NWFSC-135

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Exposure to a Mixture of Toxic Chemicals:

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Reference this document as follows:

Mongillo, T. M., G. M. Ylitalo, L. D. Rhodes, S. M. O'Neill, D. P. Noren, and M. B. Hanson. 2016. Exposure to a mixture of toxic chemicals: Implications for the health of endangered Southern Resident killer whales. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-135, 107 p. doi:10.7289/V5/TM-NWFSC-135

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Executive Summary

The distinct population segment (DPS) of Southern Resident killer whales (*Orcinus orca*) was listed as endangered under the Endangered Species Act (ESA) on 18 November 2005. The Southern Residents regularly occur in the inland waters of Washington and British Columbia during late spring, summer, and early fall. Less is known about their movements in the winter, but they occur in coastal waters from California to southeast Alaska. Many studies have indicated that they primarily consume Chinook salmon (*Oncorhynchus tshawytscha*). Several major threats were identified—both in the final determination to list the Southern Resident killer whale DPS as endangered, and in the Southern Resident killer whale recovery plan—one of which was exposure to high levels of organochlorine contaminants and increasing levels of emerging contaminants.

The primary objectives of this Technical Memorandum are to review the contaminants that may pose a risk to the Southern Resident killer whales and to discuss the health implications of exposure to these contaminants. In this report, we focus on three persistent organic pollutants (POPs): polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichlorodiphenyltrichloroethane (DDT) and its metabolites. We focus on these three POPs because they are found at relatively high levels in the whales and may cause adverse health effects. We also describe what is currently known about the whales' geographic distribution and diet, as well as contaminant levels measured in their prey. We review the factors that influence contaminant bioaccumulation and the development of biomarkers for exposure and toxicity. Lastly, we highlight data gaps and make recommendations for future studies.

Adult killer whales are primarily exposed to POPs through the ingestion of prey. Based on the importance of Chinook salmon as prey, and their elevated POP levels compared to other salmonids, they are likely a significant source of contaminants to the Southern Residents. Various adverse health effects have been associated with exposures to PCBs, PBDEs, and DDTs in humans, laboratory animals, and wildlife. These POPs have the ability to cause endocrine disruption, reproductive disruption or failure, immunotoxicity, neurotoxicity, neurobehavioral disruption, and cancer. The average concentration of blubber summed PCBs (ΣPCBs) in male Southern Resident killer whales sampled between 2004 and 2013 was 45,000 ± 31,000 ng/g lw (lipid weight), which exceeds a health effects threshold in harbor seals (*Phoca vitulina*). Average blubber ΣPBDEs in sampled Southern Residents were 4,800 ± 3,500 ng/g lw, with most individuals exceeding the levels associated with altered thyroid hormone levels in post-weaned and juvenile gray seals (*Halichoerus grypus*). Although there has been no report in the literature on a marine mammal health effect threshold for DDTs, ΣDDTs levels in the blubber of Southern Residents were high, and ranged from 1,200 to 210,000 ng/g lw.

The high levels of POPs in the Southern Residents have health implications that are influenced by interactions with other stressors, including the abundance of their prey. Reduced prey availability can cause a whale to draw on lipid reserves stored in its blubber, mobilizing POPs into circulation, where they have the potential to cause a toxic response. Therefore, nutritional stress from reduced Chinook salmon populations may act synergistically with high POP levels in Southern Residents and result in deleterious health effects.

The potential for adverse health effects from POP exposure in an individual killer whale is also influenced by the timing of the exposure. For example, killer whale calves may be more susceptible to POP-induced endocrine disruption because they are exposed during critical stages of their development, when healthy growth and development rely on normal levels of hormones. The effects from exposure during this vulnerable life stage could include alterations to the individual's metabolism, impeded growth and development, delayed or premature physical or sexual maturity, reduced future fecundity, or reduced perinatal survival. Contaminant exposure during neurodevelopment can also reduce learning or impair memory. With this reduced ability to learn, a killer whale's capacity to successfully forage and interact with other pod members could be affected.

High exposure at a young age can also compromise the immune system and increase disease susceptibility. For example, high POP exposure during nursing can cause a significant reduction in antibody response and create a greater predisposition for opportunistic or secondary infections. Because infectious diseases are a large source of morbidity and mortality in marine mammals, a compromised immune system bodes poorly for the overall health status, well-being, and anticipated life expectancy of individual killer whales. In the Appendices, we report and identify pathogens and infections for wild and public-display killer whales, noninfectious diseases for killer whales, and infectious diseases for sympatric marine mammals.

Health effects from exposure to PCBs, PBDEs, and DDTs should not be considered in isolation. Killer whales are exposed to a mixture of pollutants, some of which may interact synergistically and enhance toxicity, influencing the health of the Southern Residents. Although it is difficult to predict health effects from mixture interactions, it is important to predict the toxicity of such mixtures; disregarding the interactive effects may underestimate risk to an individual or to the population. Furthermore, we also stress the importance of establishing the impact on the health of killer whales of the transformed by-products, or metabolites, of the pollutants. The practice of examining only high doses of POPs may also underestimate the risk to the killer whales. Endocrine disruptors can produce non-linear dose–response effects and interact at lower doses than would occur with the isolated chemicals. Therefore, even low concentrations of persistent pollutants, when combined, have the potential to cause adverse effects in Southern Residents.

Progress has been made in identifying potential effects from exposure to contaminants in other species. However, in order to draw meaningful conclusions about the health of killer whales, some uncertainties and data gaps still need to be addressed. In this report, we provide summaries of the projects currently in progress and recommendations for future work that can guide management actions to advance the recovery of Southern Resident killer whales. These recommendations include collecting better data on toxicant levels in the whales and their prey, examining the exposure of calves to toxicants, describing transfer dynamics, understanding the role of prey availability in the mobilization of toxicants, developing more biomarkers of exposure and toxicity, and conducting studies to assess health effects in the whales. We consider these recommendations an important step toward evaluating the current and future health of this endangered species.

Acknowledgments

We would like to acknowledge the following people who reviewed this report and provided valuable feedback: Lynne Barre, Stephen Raverty, Pete Schroeder, Jessica Lundin, Chris Yates, and Alison Agness. We would also like to thank Tina Loucks-Jaret and Kailey Genther for their additional editorial reviews.

Introduction and Objectives

Persistent organic pollutants (POPs) are a family of toxic chemicals that are lipophilic (i.e., fat soluble), resistant to environmental degradation, and can increase in concentration up the food chain (biomagnify). Because POPs are lipophilic, they are largely stored in fatty tissues in the body, such as marine mammal blubber, and can become mobilized during times of nutritional stress, illness, or during reproductive processes such as pregnancy or lactation. They are cleared from the body via several pathways, including metabolism (conversion to another product) and elimination via urine and feces or transplacental and/or lactational transfer. The ability for POPs to biomagnify means species that occupy positions at the top of the food chain can accumulate large amounts of these compounds throughout their lives and have body burdens that are several orders of magnitude greater than species that feed at lower trophic levels. At certain concentrations and mixtures, these pollutants have been associated with reproductive impairment (Reijnders 1986, Subramanian et al. 1987, Reddy et al. 2001, Schwacke et al. 2002), immunotoxicity (de Swart et al. 1996, Fonnum et al. 2006), endocrine disruption (de Boer et al. 2000, Legler and Brouwer 2003, Darnerud 2008, Legler 2008), neurotoxicity (Darnerud 2003, Viberg et al. 2003, Viberg et al. 2006, Darnerud 2008), and cancer in humans and wildlife (Ylitalo et al. 2005, Bonefeld-Jørgensen et al. 2011).

The distinct population segment (DPS) of Southern Resident killer whales (*Orcinus orca*) are long-lived, upper trophic-level predators that seasonally feed on prey that are in relatively close proximity to industrial discharge, urban drainage, and agricultural run-off areas where POPs can be highly concentrated. Consequently, they have high contaminant levels relative to other populations of killer whales. In 2001, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) categorized the Southern Residents as endangered. On 18 November 2005, the Southern Resident killer whales were listed as endangered under the U.S. Endangered Species Act (NMFS 2005) and a recovery plan was completed in 2008 (NMFS 2008). The Southern Resident killer whale population recently totaled fewer than 80 individuals in three distinct pods (J, K, and L). They consume a variety of fish species, but Chinook salmon (*Oncorhynchus tshawytscha*) are identified as their primary prey during spring, summer, and fall (Ford and Ellis 2006, Hanson et al. 2010, Ford et al. 2016).

Since the mid-1970s, the geographic range of Southern Residents has been determined primarily from opportunistic re-sightings of photo-identified individuals, and occasionally from strandings. In general, these pods have distinct summer/fall and winter/spring seasonal patterns. There is also seasonal variation between pods. While summer locations are well known (primarily inland and coastal waters of Washington and British Columbia), less is known about fall, winter, and spring. Passive autonomous acoustic recorders have recently provided more information on the seasonal occurrence of these pods along the West Coast of the United States (Hanson et al. 2013). In addition, satellite-linked tags were recently deployed in winter months on members of J, K, and L pods. Results were consistent with previous data, but provided much greater detail, showing J pod's wide-ranging use of inland waters, and K and L pods' extensive movements in U.S. coastal waters (NWFSC unpubl. data).

Critical habitat was designated in 2006 and includes approximately 2,560 square miles of inland Washington waters in three specific areas: 1) the Summer Core Area in Haro Strait and the waters around the San Juan Islands, 2) Puget Sound, and 3) the Strait of Juan de Fuca (NMFS 2006). Water quality to support growth and development was identified as a feature of the critical habitat areas. In January 2014, NOAA Fisheries received a petition from the Center for Biological Diversity to revise the critical habitat designation based on new information about the whales' habitat use along the coast and the consideration of in-water sound as an element of habitat. In February 2015, a 12-month finding was issued that identified next steps for collecting and analyzing data, and for developing a proposed rule to revise critical habitat, expected in 2017 (80 FR 9682; 24 February 2015).

Several factors currently pose a risk to the Southern Residents, including exposure to high levels of organochlorine contaminants and increasing levels of emerging contaminants (e.g., brominated flame retardants); reduced prey availability, size, and quality; noise pollution and vessel activity; infectious disease; and oil spills (NMFS 2008). These factors most likely interact; for example, disease may be the ultimate result of chronic exposure to or increased levels of contaminants that can reduce resistance to pathogens in the marine environment.

The objectives of this Technical Memorandum are:

- to review the contaminants that may pose a health risk to the Southern Resident killer whales;
- to describe what is currently known about the Southern Residents' geographic distribution;
- to review the diet and the POP levels measured in the Southern Residents' prey;
- to describe the factors that influence bioaccumulation and affect the variability of POP levels measured in Southern Resident killer whales and other marine mammals;
- to describe the potential adverse health effects of isolated POPs (PCBs, PBDEs, and DDTs);
- to review the development of biomarkers for exposure and toxicity;
- to describe the interactive effects of exposure to a mixture of POPs;
- to discuss the implications for the health of the Southern Residents from exposure to POPs;
- to highlight data gaps and make recommendations for future studies.

Contaminants of Concern

In 2008, NMFS developed the Southern Resident killer whale recovery plan. In the plan, a number of environmental contaminants that may pose a health risk to killer whales were highlighted (Table 1). In this section, we provide background information on polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichlorodiphenyltrichloroethane (DDT) and its metabolites. Although levels of PCBs and DDTs have dramatically decreased in environmental samples since their production ban in the mid-1970s (Mearns 1988, Lieberg-Clark et al. 1995, Calambokidis et al. 2001, Rigét et al. 2010), these compounds continue to be found in substantial levels in marine biota around the world, including killer whales and other cetaceans from the eastern North Pacific (Ylitalo et al. 2001, Kajiwara et al. 2006, Krahn et al. 2007a, Kajiwara et al. 2008, Krahn et al. 2009, Ylitalo et al. 2009, Jepson et al. 2016). Levels of PBDEs were increasing almost exponentially in several marine species between the 1980s and early 2000s (Ikonomou et al. 2002, Lebeuf et al. 2004, Ross et al. 2013), causing a concern among scientists for many food webs. More recently, it appears that several species are experiencing declining or stabilizing PBDE concentrations (Elliott et al. 2005, Law et al. 2010, West et al. 2011, Ross et al. 2013), likely because of the decrease in production and use of certain PBDE technical mixtures. However, due to the presence of PBDEs in currently used products, they will continue to be delivered to the environment, contaminating the aquatic food web for many more years.

These three classes of POPs (PCBs, PBDEs, and DDTs) are the focus of this report, as they are found at relatively high levels in Southern Resident killer whales, their prey, and their environment. Adverse health effects may be exacerbated when combinations of these POPs are present in organisms. We also briefly discuss other toxic chemicals of concern, including polycyclic aromatic hydrocarbons (PAHs) and other trace elements that may pose a risk to the killer whales. Although there are several toxic chemicals of concern (e.g., dioxins, furans, personal care products, other endocrine disruptors, etc.), they are not discussed here as they are outside the scope of this Technical Memorandum.

Table 1. Persistent pollutants that may pose a risk to Southern Resident killer whales.[a]

Pollutant(s)	Use and/or Source	Persistent	Bioaccumulative	Risks
PCBs (*polychlorinated biphenyls*)	electrical transformer and capacitor fluid with limited use in North America; enters environment from runoff, spills, and incineration	yes	yes	reproductive impairment, skeletal abnormalities, neuro- and immunotoxicity, terato- and carcinogenicity, endocrine disruption
PBDEs (*polybrominated diphenyl ethers*)	two of three product PBDEs banned in Europe; same two products withdrawn from North American marketplace in 2005, one (deca-) product still used globally in electrical components, backings of televisions and computers, textiles and vehicle seats; ubiquitous in environment	yes	yes	endocrine disruption, liver and thyroid function impairment, autoimmunity induction, immunosuppression; impacts on lung and neural development
DDT (*dichlorodiphenyl-trichloroethane*)	pesticide still used in some countries, currently banned in North America; persists in terrestrial runoff 30 years post-ban, enters atmosphere from areas where still in use	yes	yes	reproductive impairment, immunosuppression, adrenal and thyroid effects
dioxins, furans	burning of salt-laden wood, municipal incinerators, residential wood and wood waste combustion, runoff from sewage sludge, wood treatment; by-product of chlorine bleaching, wood product processing, and incomplete combustion; pulp and paper mills	yes	yes	thymus and liver damage, birth defects, reproductive impairment, endocrine disruption, immunotoxicity, cancer
PAHs (*polycyclic aromatic hydrocarbons*)	fuel combustion, aluminum smelting, wood treatment, oil spills, metallurgical and coking plants, pulp and paper mills	yes	relatively low potential; may depend on molecular weight	carcinogenic and cardiac dysfunction, developmental neurotoxicity, reproductive dysfunction, immunotoxicity, eggshell thinning
PFOs (*perfluorooctane sulfonate*)	stain, water, and oil repellent (until recently included in Scotchgard), fire-fighting foam, fire retardants, insecticides and refrigerants; ubiquitous in environment	yes	yes, in blood, liver, kidney, and muscle	promotion of tumor growth

[a] Source: Updated from NMFS (2008).

Table 1 continued. Persistent pollutants that may pose a risk to Southern Resident killer whales.[a]

Pollutant(s)	Use and/or Source	Persistent	Bioaccumulative	Risks
TBT, DBT (*tributyltin, dibutyltin*)	antifoulant pesticide used on vessels	yes	yes	unknown, but recently associated with hearing loss
PCPs (*polychlorinated paraffins*)	flame retardants, plasticizers, paints, sealants, and additives in lubricating oils	yes	yes	endocrine disruption
PCNs (*polychlorinated naphthalenes*)	ship insulation, electrical wires and capacitors, engine oil additive, municipal waste incineration, and chloralkali plants; contaminant in production of technical PCBs	yes	yes	endocrine disruption
APEs (*alkylphenol ethoxylates*)	detergents, shampoos, paints, pesticides, plastics, pulp and paper mills, textile industry; found in sewage effluent and sediments	moderate	moderate	endocrine disruption
PCTs (*polychlorinated terphenyls*)	fire retardants, plasticizers, lubricants, inks and sealants; enters environment in runoff	yes	yes	endocrine disruption and reproductive impairment

References: Primarily Grant and Ross (2002), but also Lindstrom et al. (1999), Hooper and McDonald (2000), Kannan et al. (2001), Hall et al. (2003); Legler and Brouwer (2003), Van de Vijver et al. (2003), Rayne et al. (2004), Song et al. (2005), Ylitalo et al. (2005), Darnerud (2008), Legler (2008), Fernie et al. (2009), and Kodavanti et al. (2010).

[a] Source: Updated from NMFS (2008).

Persistent Organic Pollutants (POPs)

POPs enter fresh and marine waters and sediments from numerous direct and indirect sources. For example, POPs can enter a local environment through poorly maintained hazardous waste sites, illegal dumping, leaks from electrical transformers, disposal of consumer products containing POPs, and burning of some wastes in municipal and industrial incinerators. Point source (or direct) pollution is pollution that is discharged directly into the marine environment from an identifiable source, such as an outfall from municipal and industrial wastewater treatment plants, or pulp and paper mills. In contrast, non-point source (or indirect) pollution does not have an identifiable source. Examples of non-point sources include surface runoff and atmospheric deposition. Oceans act as a repository for domestic and industrial wastes, and significant concentrations of POPs have been measured in ocean sediments and biota. Recently, a multiphase project was initiated by multiple agencies to assess toxic chemical loadings from point and non-point sources into the whales' critical habitat. The 10 major pathways for pollution are from surface runoff, groundwater, rivers and streams, atmospheric deposition, wastewater, combined sewer overflows, oil spills, migrating biota such as salmon, inflow from the Pacific Ocean, and contaminated sediment (Hart Crowser, Inc. et al. 2007).

POPs are found throughout the world, regardless of where they were produced or used, because they can be transported through several pathways. They are typically concentrated near populated areas of high human activity and industrialization. The highest levels of these compounds have been measured in species from the Northern Hemisphere, specifically in industrialized Asia, North America, and Europe (Houde et al. 2005). The dispersion of POPs in the environment depends on the pollutant's physicochemical properties, with long-range and global transport occurring via oceanic and riverine transport and atmospheric deposition (Iwata et al. 1993, Grant and Ross 2002, Houde et al. 2005). These physicochemical properties include persistence (measured by the environmental half-life), vapor pressure, water solubility, and bioaccumulation potential (expressed in terms of the octanol–water partitioning coefficient K_{ow}; Grant and Ross 2002).

Once the contaminants enter the marine environment, several factors influence the retention and flushing of these compounds in the local environment, including ocean circulation and the physical structure of the basin. For example, Puget Sound is a deep-water fjord with several sills that restrict mixing and inhibit both ocean inflow and the outflow of toxic chemicals. As a result, POPs that enter the Puget Sound basin have long residence times, resulting in an increase in contaminant exposure and bioaccumulation in local food webs. Additionally, many species are known to exhibit a high degree of residency within Puget Sound (e.g., there are several resident populations of fish in Puget Sound, including Pacific herring [*Clupea pallasii*] and Chinook salmon), resulting in more fish being exposed to more contaminants (West et al. 2008, O'Neill and West 2009). Thus, the Puget Sound ecosystem and food webs are especially susceptible to toxic input due to their proximity to urban areas and the combination of the hydrological isolation of Puget Sound and the biological isolation of its resident species (Collier et al. 2007, West et al. 2008, O'Neill and West 2009).

Polychlorinated Biphenyls (PCBs)

PCBs are synthetic organic chemicals with 209 potential congeners, or forms. The chemical structure of PCBs consists of from one to ten chlorine or hydrogen atoms attached to biphenyl, a molecule composed of two benzene rings (Figure 1). PCB congeners are numbered according to the International Union of Pure and Applied Chemistry (IUPAC) system (i.e., a systematic method of naming organic chemical compounds). They were designed for chemical stability and were historically used for transformers and capacitors. The manufacture of PCBs was banned in the U.S. in 1979 with the recognition of their global distribution, persistence in nature, and potential for adverse health effects on wildlife and humans.

The number and position of chlorine atoms influence the volatility, persistence, aqueous solubility, bioaccumulation potential, and toxicity of the PCB congeners. For example, the more chlorinated PCB congeners tend to be more environmentally persistent and less volatile than the less chlorinated congeners (Grant and Ross 2002). In addition, the more chlorinated PCBs tend to have a higher bioaccumulation potential and resist metabolic degradation more than the less chlorinated PCBs that may readily metabolize (Grant and Ross 2002). The toxic endpoint of a PCB congener can depend on the planarity of the molecule or the position of the chlorine atoms (Silkworth and Grabstein 1982). The planar PCB congeners, such as those with non-ortho chlorine substitution, are structurally similar to dioxin. Dioxins are highly toxic and can cause reproductive impairment, disruption to the immune system, endocrine disruption, developmental problems, and also cause cancer (WHO 2010). Thus, the more acutely toxic PCB congeners are those that are more dioxin-like. However, further research is revealing that, though the non-dioxin-like PCB congeners—the ortho-substituted congeners—have different toxic endpoints, they are no less concerning. For example, researchers have reported that some of the non-dioxin-like PCBs can interfere with hormone-regulated processes (Bonefeld-Jørgensen et al. 2001, Oh et al. 2007) and enhance developmental neurotoxicity (Fischer 2008) and cytotoxicity (Pellacani et al. 2014). In general, some PCBs persist in nature, bioaccumulate in upper trophic level species, resist metabolic degradation, and may cause adverse health effects in many species.

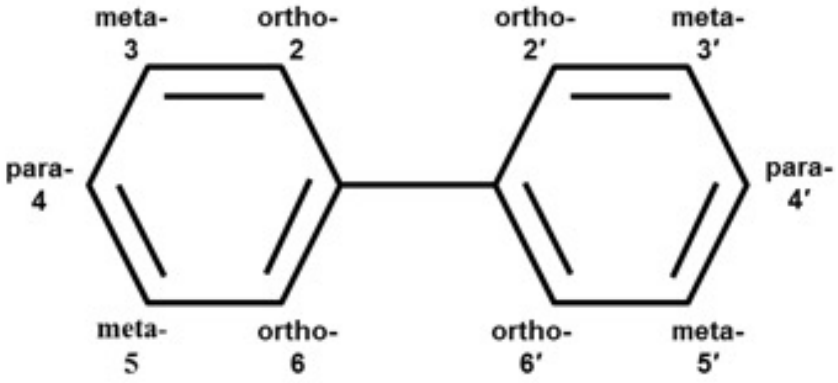

Figure 1. Chemical structure of PCBs. The numbers assigned to the carbon atoms indicate the potential positions of the chlorine atoms on the benzene rings. The meta-, ortho-, and para- labels describe the placement of the chlorine atoms.

Although many areas in the Pacific Northwest lack information on both historical and current levels of PCB contamination, there are some data that reveal regional PCB trends in sediment. Sediments in Washington and British Columbia have been routinely monitored for contaminant exposure since the early 1900s. These data indicate a trend in PCB contamination levels similar to the global production of PCBs. PCBs reached a peak in global production by 1970 (Breivik et al. 2002). PCB trends in sediment in Puget Sound and the Strait of Georgia showed a steady increase in concentration beginning in 1930 that reached peak levels in the early 1960s (Lefkovitz et al. 1997, Johannessen et al. 2008). The peak PCB levels in the sediment at that time were close to four times the levels of PCB sediment contamination in the 1990s (Lefkovitz et al. 1997). More recently, sediment cores from the Strait of Georgia indicate a declining trend in PCB concentrations (Johannessen et al. 2008).

PCB data for some wildlife also indicate recent declines in concentrations. Since 1986, NOAA's National Status and Trends Program has been monitoring PCBs and other contaminants in mussels and oysters around the U.S. coastline, including 17 sites within Washington State. Because these organisms are filter feeders, their tissue is considered an indicator for local water quality conditions. PCB concentrations in mussels in Puget Sound have been slowly declining; however, in general they remain above the national average (PSAMP 2007). Puget Sound harbor seals (*Phoca vitulina*) have also experienced declines in PCB concentrations beginning in the 1970s (Calambokidis et al. 1999). More recent data indicate that PCB concentrations in harbor seals have declined by 81% from 1986 to 2003 (Ross et al. 2013). Similar to the local declines of PCBs in both the sediment and wildlife, a global decline of PCB levels has been observed. For example, PCB levels in Swedish women's breast milk were shown to decrease by 30% from 1972 to 1997 with a half-life of 14 years (Norén and Meironyté 2000).

Not all species in Puget Sound, however, have experienced a decline in PCB levels. For instance, PCB concentrations in Pacific herring throughout Puget Sound remain high and have lacked a declining trend (West et al. 2011). PCB concentrations in English sole (*Parophrys vetulus*) from most urban locations have also lacked a declining trend and exceed harmful effects thresholds, indicating that PCBs are still a concern in Puget Sound (West et al. 2011).

Polybrominated Diphenyl Ethers (PBDEs)

PBDEs are another class of synthetic organic compounds with 209 possible congeners. The chemical structure consists of two phenyl rings connected by an ether bond with from one to ten bromine or hydrogen atoms attached (Figure 2). PBDEs are structurally similar to PCBs and have similar physicochemical properties. PBDE congeners are numbered according to the IUPAC system that was originally designed for PCBs. In general, several PBDEs are lipophilic, persistent in the environment, and accumulate in ocean sediments because of their high binding affinity to particles (de Wit 2002). The lighter PBDEs are highly bioaccumulative, more readily biomagnify in aquatic and terrestrial food webs, are more toxic than the heavier congeners, and present an increased risk to the health of marine mammals (Siddiqi et al. 2003, Ross et al. 2009). In contrast, the uptake of the larger congeners (e.g., BDE-209) is restricted by particle-binding (Ross et al. 2009), although these heavier weighted congeners are still observed in upper trophic level species

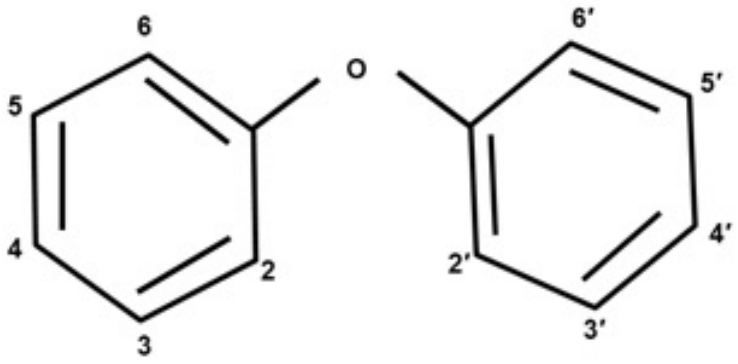

Figure 2. Chemical structure of PBDEs. The numbers assigned to the carbon atoms indicate the potential positions of the bromine atoms on the phenyl rings.

(e.g., harbor seals; Shaw et al. 2007). All of the PBDE homologue groups, or groups of congeners with the same number of bromine atoms, appear to be environmentally persistent (Ross et al. 2009) and may be more persistent than PCBs (de Boer et al. 1998).

PBDEs have been identified as a growing concern. They are ubiquitous, with increasing levels found in various matrices, including surface water, sewage sludge, sediment, air, and biota (Hale et al. 2003, Hites 2004). Since the 1960s, PBDEs have been used as additive flame retardants in many products, including electronics, textiles, and plastics. Additive flame retardants are mixed into products, which can cause them to leach more readily into the environment (de Boer et al. 2000, de Wit 2002) than reactive flame retardants, which are covalently bound to their product and thus less likely to leach into the environment. PBDE production has three commercial mixtures (penta-, octa-, and deca-BDEs), and each mixture comprises a combination of PBDE congeners that have been used in a variety of products. For example, the main constituents of the penta-mixture include BDE-47, -99, and -100, and to a lesser degree congeners -153, -154, and -85 (Hale et al. 2003). These congeners, as well as -209, are considered of particular importance and concern. Almost half of the production of the deca- and octa- forms, and over 95% of the penta-products, has occurred in North America (Hale et al. 2003, Hites 2004). As a result, PBDE levels in humans in the United States are from 10 to 100 times greater than elsewhere (Schecter et al. 2003).

Because of health concerns, manufacturers in the United States voluntarily stopped producing the penta- and octa- mixtures by the end of 2004. In January 2006, the Washington State Department of Ecology (Ecology) and the Washington State Department of Health (Health) issued a Final PBDE Chemical Action Plan (Ecology and Health 2006), recommending that the Washington State Legislature prohibit the sale, manufacture, or distribution of new products containing penta- or octa-BDE in Washington State. In 2007, the Washington State Legislature adopted RCW 70.76; under this law, no person may manufacture, knowingly sell, offer for sale, distribute for sale, or distribute for use in this state nonedible products containing PBDEs, effective January 2008. The exemptions from this prohibition are described in RCW 70.76.020, and include products containing deca-BDE; the sale or distribution of any used vehicle or vehicle parts manufactured before January 2008; and equipment used primarily for military or federally funded space program applications, medical devices, and certain recycled materials. In 2010, Environment

Canada and Health Canada published a Final Revised Risk Management Strategy for PBDEs that: 1) prohibits the use, sale, offer for sale, and import of all PBDEs; 2) prohibits PBDEs in products; 3) describes a commitment to a voluntary phase-out for deca-BDE; 4) monitors PBDEs in the Canadian environment, including landfills and at wastewater treatment plants; 5) develops Federal Environmental Quality Guidelines; and 6) develops a strategy that includes continuing research and implementing risk-management activities. Effective January 2011, deca-BDE was prohibited in televisions, computers, and furniture following the identification of safer alternatives. Deca-BDE (a commercial mixture of PBDEs that contains 97% of congener BDE-209) now accounts for all PBDE production in North America, and is primarily used in textiles and added to various plastic polymers (Shaw and Kannan 2009). Consequently, the heavier PBDE congeners compose a major proportion of the total PBDEs found in the environment, and can become a source of the lighter, more persistent, and more toxic congeners (Johannessen et al. 2008, Ross et al. 2009).

Specific data are limited for PBDE levels in Washington State. The environmental levels of the less-brominated congeners appear to have surpassed PCBs in some areas in North America, including in air, water, sewage sludge, and sediment (Hale et al. 2003, Ross et al. 2009). Time trend studies indicate an increase of PBDEs in biota since the 1970s (de Wit 2002, Shaw and Kannan 2009). PBDE concentrations in harbor seals from Puget Sound increased exponentially, with a doubling time of 3.1 years, from 1984 to 2003, but appeared to drop in 2009 (Ross et al. 2013). Pacific herring also appear to have PBDE levels that are declining or remaining stable, whereas English sole from urban basins showed no declining trend in PBDE concentrations (West et al. 2011). PBDEs in great blue heron (*Ardea herodias*) eggs from the Fraser River estuary and double-crested cormorant (*Phalacrocorax auritus*) eggs from the Strait of Georgia increased exponentially until the mid-1990s, followed by a brief decrease in concentrations (Elliott et al. 2005).

Dichlorodiphenyltrichloroethane (DDT and Metabolites)

DDTs have a ubiquitous distribution and are found in air, water, soil, and biota. DDTs are extremely persistent in nature, semi-volatile, highly stable, have low water solubility, biomagnify in the food chain, and are highly toxic to aquatic organisms. These organochlorines are produced by the reaction of chloral with chlorobenzene in the presence of a catalyst, sulfuric acid (Figure 3). DDTs have 14 carbon atoms, nine hydrogen atoms, and five chlorine atoms (Figure 3). DDE and DDD are the major metabolites of DDT, and are also highly persistent. In fact, p,p'-DDE is the most abundant DDT metabolite found in killer whale blubber, accounting for approximately 80% of the summed DDTs (ΣDDTs) measured between 1994 and 1999 in Alaska resident killer whales and 86% of the ΣDDTs in transient killer whales (Ylitalo et al. 2001).

DDT was first used as an insecticide in the late 1930s and during World War II on troops and civilians to control insects that transmit diseases such as malaria and typhus. After the war, the use of DDT increased to include controlling pests in commercial and agricultural areas, forests, homes, and gardens. By 1972, more than 675,000 tons of DDT had been applied domestically in the United States, with 1959 being the peak year for DDT use (40,000 tons; EPA 1972).

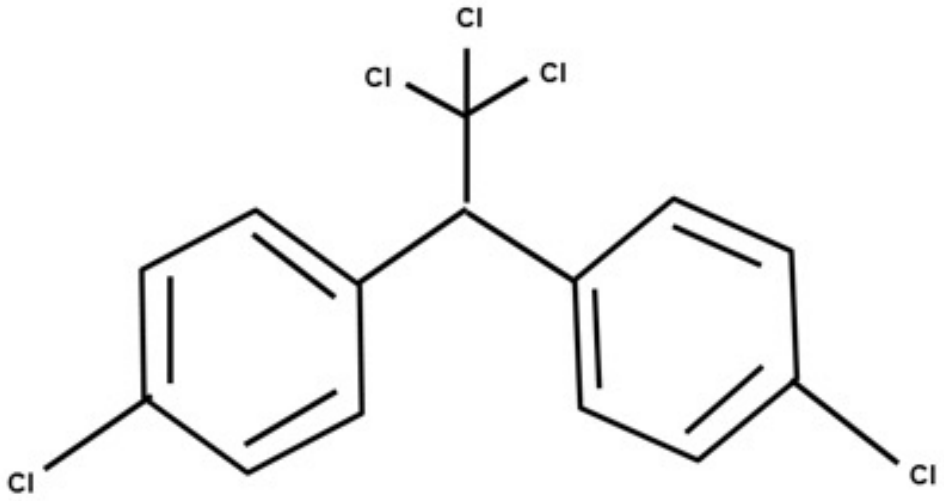

Figure 3. Chemical structure of DDT. DDT contains 14 carbon atoms, nine hydrogen atoms, and five chlorine (Cl) atoms.

Reproductive dysfunction, as well as eggshell thinning, was linked to exposure to DDTs in various bird species (reviewed in Fry 1995). Because of its toxic effects on wildlife and laboratory animals and its potential health risks to humans, the United States and several other countries banned general usage of DDT in the 1970s. Production of this insecticide was stopped by 1985 in the United States; its worldwide decrease in use, the development of alternative pesticides, public awareness of its potential toxicity to humans and wildlife, and insect resistance were all contributing factors. In 2001, the Stockholm Convention ratified that the use of DDT be restricted to controlling disease vectors under the guidance of the World Health Organization (Stockholm Convention 2001). Thus, this pesticide continues to be used in certain areas of the world (e.g., Africa) to control for malaria and other insect-borne diseases (Biscoe et al. 2005).

DDTs frequently co-occur in the marine environment with PCBs and other POPs. Many of these compounds possess certain chemical properties (e.g., stability, low flammability, lipophilicity) that not only made them effective pesticides and industrial compounds, but also make them highly persistent, bioaccumulative environmental contaminants. In Puget Sound, contemporary levels of DDTs in sediments and marine fish are generally lower than PCB concentrations (Brown et al. 1998, Calambokidis et al. 2001, West et al. 2008). For example, Johnson et al. (2007) found that concentrations of ΣPCB measured in whole bodies of outmigrating juvenile Chinook salmon captured in Puget Sound were more than two times higher than the ΣDDT values. In contrast, contemporary levels of DDTs are higher than PCB concentrations in sediments and marine biota from central and southern California (Jarvis et al. 2007, Blasius and Goodmanlowe 2008, Kimbrough et al. 2008). These higher levels of DDTs in California (the "California signature") are due to heavy agricultural use of this compound before its ban in the 1970s, as well as to the long-term significant discharges of DDTs from a manufacturing plant (Eganhouse et al. 2000, Bay et al. 2003). Although bivalves in southern California had the highest ΣDDT concentrations compared to bivalves sampled elsewhere along the continental U.S., average concentrations are declining (Sericano et al. 2014).

Other Toxic Chemicals of Concern

Polycyclic Aromatic Hydrocarbons (PAHs)

There is a suite of toxicants, including polycyclic aromatic hydrocarbons (PAHs), that frequently occur in the marine environment and may affect Southern Resident killer whales. PAHs are a class of compounds that are derived primarily from petroleum products or the combustion of these products, and can enter the marine environment directly (e.g., oil spill) or indirectly (e.g., stormwater runoff, atmospheric deposition). Concerns have been raised over the effects of exposure to PAHs on marine organisms, both because of the worldwide use of fossil fuels (Geraci and St. Aubin 1990, Peterson et al. 2003) and the occurrence of oil spills in areas that support populations of marine life. Marine biota can take up PAHs present in the environment via a number of routes (e.g., dermal absorption, inhalation, consumption of contaminated prey or sediment; Meador et al. 1995). However, the persistence of these compounds in tissues and body fluids of exposed marine organisms varies depending upon the rates of uptake, metabolism, and elimination (Krahn and Stein 1998). Vertebrates, such as fish and marine mammals, quickly metabolize PAHs into more polar forms that are then excreted into urine or secreted into bile for rapid elimination via feces (Roubal et al. 1977, Krahn et al. 1984, Varanasi et al. 1989). However, some of the PAH intermediates formed during metabolism can be more toxic, and may pose a greater health risk than the parent PAHs (Varanasi et al. 1989).

Exposure to PAHs in aquatic vertebrates, including the potential prey of Southern Residents, has been linked to developmental deformities, altered growth, adrenal dysfunction, immunosuppression, and hepatotoxicity (Heintz et al. 2000, Arkoosh et al. 2001, Incardona et al. 2004, Schwartz et al. 2004, Meador et al. 2006, Reynaud and Deschaux 2006, Carls and Meador 2009, Incardona et al. 2012). In marine mammals, studies examining the effects of oil exposure have demonstrated them to be both physiological (e.g., fouling of pelage or baleen) and toxicological (e.g., fetal distress and in utero pneumonia, conjunctivitis, increased plasma cortisol levels, neural damage, pulmonary emphysema, adrenal gland and lung lesions, gastric erosions and ulcers, and hepatocellular necrosis; Geraci and St. Aubin 1990, Spraker et al. 1994, O'Hara and O'Shea 2001, Venn-Watson et al. 2015, Colegrove et al. 2016). However, information on the effects of inhalation of PAHs by marine mammals is lacking.

Trace Elements

Unlike the persistent pollutants described above, trace elements (often referred to as metals) are naturally found in the environment. Some are essential to animals' nutrition. Most metals, like persistent pollutants, settle to the ocean floor, where they can accumulate in sediment. Human activities can increase the concentrations, and metals can become toxic at certain exposure levels. Therefore, areas with high human activity can become hotspots of multiple toxic chemicals.

Marine mammal exposure to mercury, cadmium, and lead has been the focus of several studies due to the known toxicity of these metals to humans and other wildlife, with effects such as damage to the central nervous system, skeletal deformities, kidney lesions, and kidney or liver damage, as well as carcinogenic, mutagenic, and teratogenic effects (O'Shea 1999, Das et al. 2003). Although elevated mercury has been associated with immunosuppression in harbor seals (Das et al. 2008), little information is known about the toxic effects of heavy metals in marine mammals.

The distribution or storage of heavy metals in marine mammals is dependent on the metal. In general, heavy metals are found in the liver, kidneys, muscles, and bones (O'Shea 1999, Reijnders and Aguilar 2002, Das et al. 2003). Concentrations and types of heavy metals measured in marine mammals are primarily influenced by the levels found in their prey and by their geographical range, as well as the age and sex of the individual. For example, marine mammals that feed on squid can be exposed to higher levels of cadmium, copper, and zinc, because squid have the ability to retain these elements (Reijnders and Aguilar 2002). Currently, there are few metals data available on Southern Resident killer whales (Anulacion et al. in prep.). Some metals may transfer from mother to offspring during gestation and lactation, although not to the same degree as the persistent organic pollutants.

Some marine mammals (particularly from the northern arctic regions) appear to tolerate high levels of some metals, and are able to detoxify them through several processes. For example, cadmium and mercury can combine with selenium or metallothionein (MT, a protein molecule) to mitigate the toxic effects of exposure (Meador et al. 1999, Rooney 2007, Klaassen et al. 2009). These new complexes (mercury and selenium or cadmium and MT) in the liver or kidneys change the metals into non-toxic forms and mitigate their toxic effects (Klaassen et al. 2009). This detoxification mechanism appears to be species-specific. For example, unlike in sperm whales, that did not show an obvious relationship between mercury and selenium, pilot whales demonstrated a strong correlation between mercury and selenium, with an almost fourfold higher molar ratio than that found in the sperm whales (Nielsen et al. 2000).

Geographic Distribution of Southern Resident Killer Whales

The summer range of Southern Residents has been fairly well defined based on numerous sightings over the past 34 years (Heimlich-Boran 1988, Hauser et al. 2007, Whale Museum unpubl. data). All three Southern Resident pods regularly occur in the waters of the Georgia Basin (the Strait of Georgia, Haro Strait, and the Strait of Juan de Fuca) during late spring, summer, and early fall (Heimlich-Boran 1988, McCluskey 2006, Hauser et al. 2007, Whale Museum unpubl. data). For the past few years, J pod has typically returned by May, and is usually not a regular visitor until June. Similarly, K and L pods return to the inland waters and are regular visitors in late June, although K pod has sometimes not been sighted in inland waters until July. With the exception of forays of a few days to the outer coast, all three pods generally remain in the Georgia Basin through October (Hanson and Emmons 2010, Whale Museum unpubl. data). During this summer and early fall period, their movements are concentrated primarily in Haro Strait and the southern Strait of Georgia (Heimlich-Boran 1988, Felleman et al. 1991, Hauser et al. 2007). During their forays to the outer coast, the whales typically travel along the southern coast of Vancouver Island and are occasionally sighted as far west as Tofino and Barkley Sound. Southern Resident killer whales clearly have a core summer range area that is spatially separate from Northern Resident killer whales, which predominantly frequent the waters of central and northern British Columbia.

The range of Southern Residents throughout the rest of the year is not as well known. During the early fall, the movements of Southern Residents, particularly J pod, expand to include Puget Sound (Hanson and Emmons 2010, Whale Museum unpubl. data). By late fall, all three pods are seen less frequently in inland waters. These pods are typically observed to exit and enter through the Strait of Juan de Fuca, suggesting movements in the region of Vancouver Island and Washington State. In recent years, several sightings or acoustic detections of Southern Residents have been obtained off the Washington and Oregon coasts in the winter and spring (Hanson et al. 2010, Hanson et al. 2013, NWFSC unpubl. data). Although sightings on the outer coast are extremely limited, researchers have confirmed that K and L pods have traveled as far south as central California (NMFS 2008, Hanson et al. 2013) and as far north as southeast Alaska (one sighting occurred in Chatham Strait, AK; Hilborn et al. 2012). The limited range of the sightings/acoustic detections of J pod in coastal waters, the lack of coincident occurrence during the K and L pod sightings, and the results from satellite tagging in 2012–2016 (NWFSC unpubl. data) indicate J pod's limited occurrence along the outer coast and extensive occurrence in inland waters, particularly in the northern Georgia Strait. Because J pod spent very little time in coastal waters during tag deployments, we know less of its coastal distribution than we do for K and L pods. Indirect evidence of different ranges among the pods have also been gained from analyses of contaminant profiles of biopsy samples that show the "California signature" for K and L pods but not for J pod (Krahn et al. 2007a, Krahn et al. 2009). In addition, J pod has a much higher level of PBDEs, indicating consumption of prey that remain closer to urban settings (Krahn et al. 2007a, Krahn et al. 2009).

POPs in Southern Resident Killer Whale Prey

Marine mammals receive the majority of POPs from their diet. Adult Southern Resident killer whales primarily consume Pacific salmon, and therefore obtain the majority of their POPs from salmon. During the summer, resident killer whales in the Northeast Pacific Ocean frequent regions of high-relief topography along salmon migration routes, and early studies inferred salmon to be a primary prey item based on co-occurrence (Heimlich-Boran 1988). Anecdotal observations during subsequent studies indicated that salmon were common prey items for Southern Resident killer whales in the summer. Studies of stomach contents and surface predation event sampling indicated that Chinook salmon were a preferred prey item to both Northern and Southern Resident killer whales (Ford et al. 1998), although it was acknowledged that potential biases existed because of the small sample size and sampling only predation at the surface. Follow-up studies with increased sample sizes continued to indicate that Chinook salmon were a preferred prey item of resident killer whales (Ford and Ellis 2006). A more recent study with a much larger contemporary sample, as well as the genetic analysis of fecal samples, confirmed the importance of Chinook salmon in the summer diet (Hanson et al. 2010, Ford et al. 2016). Other Pacific salmon species and steelhead (*Oncorhynchus mykiss*) are sometimes consumed, but much less frequently than would be expected based on their abundance in coastal waters. Further data suggest that resident killer whales appear to be consuming older (i.e., larger) Chinook salmon (Ford and Ellis 2006, Hanson unpubl. data), indicating a high degree of selectivity (NWFSC unpubl. data). Additional genetic analyses from fish scales and tissues highlighted the seasonal importance of Fraser River Chinook salmon, particularly from the Upper and Middle Fraser, south Thompson, and Lower Fraser (Hanson et al. 2010).

Relatively little is known about prey preferences in the other seasons, but the majority of the evidence suggests that salmon are consumed year round. Coho salmon (*Oncorhynchus kisutch*) contributed to over 40% of their diet in late summer (Ford et al. 2016). Chum salmon (*Oncorhynchus keta*), sockeye (*Oncorhynchus nerka*), and steelhead were also part of their diet, but in much smaller contributions (Ford et al. 2016). There are very limited direct observation data of prey preferences in the winter and spring; however, Southern Resident killer whales have been observed feeding on Chinook salmon off the coasts of California (Black et al. 2001) and Washington (Hanson et al. 2010). Moreover, the occurrence of Southern Resident killer whales off the Washington coast coincides with spring Chinook salmon returning to the Columbia River (Zamon et al. 2007). Prey and fecal samples recently collected in winter and early spring indicate a diet dominated by salmonids, particularly Chinook salmon, with the presence of some lingcod (*Ophiodon elongatus*) and Pacific halibut (*Hippoglossus stenolepis*; NWFSC unpubl. data). Stable isotope analysis, which shows that the whales are consistently consuming high trophic level prey (Krahn et al. 2007a, Krahn et al. 2009), provides additional evidence of salmon consumption.

Bottom fish and squid (*Ommastrephes* spp.*)* have also been documented in the gut contents of stranded resident killer whales (Ford et al. 1998), and may potentially be important prey species in the winter diet of resident killer whales. Bottomfish known to be consumed include sablefish (*Anoplopoma fimbria*), English sole, and Dover sole (*Microstomus pacificus*). Boreopacific armhook squid (*Gonatopsis borealis*) were also identified in gut contents, but other large squid species (magister armhook squid [*Berrytheuthis magister*], boreal clubhook squid [*Onychoteuthis*

borealijaponica], and neon flying squid [*Ommastrephes bartramii*]) may also be important prey items. Potential but undocumented prey species include abundant fish species with similar distributions to Southern Resident killer whales, such as chub mackerel (*Scomber japonicus*), Pacific hake (*Merluccius productus*), Pacific cod (*Gadus macrocephalus*) and Pacific herring.

Various studies have measured low-to-moderate concentrations of POPs in many populations of free-ranging adult Pacific salmon, underscoring the widespread distribution of these contaminants in the aquatic environment (Table 2). Adult salmon accumulate the majority (>96%) of these POPs while feeding in the marine environment, rather than in their freshwater and estuarine habitats (Cullon et al. 2009, O'Neill and West 2009), as over 98% of their growth occurs while fish are feeding in salt water (Quinn 2005).

Overviews of PCB, PBDE, and DDT concentrations measured in Pacific salmon are presented in Table 2. The studies listed in Table 2 vary considerably in sampling design, tissue matrices analyzed, and analytical methods, and all of these factors can affect the reported POP concentrations. However, the pooled information contains sufficient data to provide general patterns of POP accumulation among fish species, and the larger geographic patterns within species. Reported POP values for Pacific salmon are limited to adults and sub-adults (i.e., those most applicable to the diet of the whales) sampled in terminal areas. Terminal areas include coastal marine waters and river mouths through which salmon migrate en route to their natal streams to spawn. Data are reported for salmon populations along the west coast of North America, from Alaska to California, to ensure adequate geographic coverage of the potential feeding ranges of Southern Residents.

Throughout their geographic range, the observed levels of POPs in adult Pacific salmon appear to be primarily determined by the marine distribution and the resulting geographic proximity of these fish to contaminated marine environments (and contaminated prey). Biological traits such as trophic status, lipid content, duration of exposure (life span and fish age), and species-specific metabolism and detoxification further exacerbate or mitigate the degree to which POPs accumulate in Pacific salmon. Species and populations of Pacific salmon vary considerably in their marine distribution and, depending on where they feed, can be differentially exposed to POPs. In general, salmon frequenting developed coastal marine waters are potentially more exposed to contaminants than those frequenting offshore waters, as coastal waters in areas with denser human populations tend to receive higher inputs from land-based sources of POPs. Remote and otherwise undeveloped offshore areas tend to be less contaminated; however, they can also receive some input of POPs via long-range atmospheric transport from more industrialized areas (Wania and Mackay 1996), or via bio-transport in the bodies of migrating animals (Blais et al. 2007).

The marine distribution of salmon varies greatly among species and populations, but in general, Chinook salmon and coho salmon have a more coastal marine distribution (preferring the continental shelf and protected inland marine waters to offshore waters; reviewed by Quinn 2005). They are therefore more readily exposed to contaminants that are present in coastal waters than other Pacific salmon species. In contrast, sockeye salmon, pink salmon (*Oncorhynchus gorbuscha*), and chum salmon have a more offshore marine distribution in

relatively uncontaminated marine waters. When sockeye salmon, pink salmon, and chum salmon enter the marine environment, they rapidly migrate northward and westward through coastal waters of North America and are found in the open waters of the North Pacific, Gulf of Alaska, and Bering Sea by the end of their first year at sea, where they reside until their return migration to their natal streams (Quinn 2005). Consequently, the amount of time they spend feeding in more contaminated coastal environments is limited, as is evident by their observed POP concentrations. Measured average concentrations of PCBs and PBDEs were highest for Chinook salmon, at 29 and 7.3 ng/g ww (wet weight), intermediate for coho salmon (14 and 0.2 ng/g ww), less for sockeye salmon (7.6 and 0.15 ng/g ww), and lowest for pink salmon and chum salmon (<3 ng/g ww and <0.2 ng/g ww), as shown in Table 2. Similarly, average DDT values were elevated in Chinook salmon and coho salmon (15.7 and 18.1 ng/g ww), lower in sockeye salmon (8.6 ng/g ww), and lowest for pink salmon and chum salmon (<2 ng/g ww).

Although Chinook salmon populations tend to be distributed along the coast, they can differ greatly in their marine distribution, even if they enter the ocean-proximate areas (Quinn and Myers 2004, Weitkamp 2010). Chinook salmon populations entering marine waters along the coast in California and Oregon encounter very different conditions than those in the Gulf of Alaska, and more northerly populations are more likely to migrate offshore than those farther south. In addition, Healey (1983) pointed out that Chinook salmon with a stream-type juvenile life history showed a greater tendency to use offshore waters than those with an ocean-type life history. A more recent analysis of Chinook salmon marine distribution confirmed this pattern, which was particularly evident in paired stream-type and ocean-type populations from the upper Columbia and Fraser rivers. Chinook salmon from coastal rivers, on the other hand, did not differ as much in marine distribution between the two life-history forms (Sharma and Quinn 2012).

The importance of marine distribution as a factor affecting POP accumulation was particularly evident for Chinook salmon populations. Although Chinook salmon generally had higher concentrations of POPs than other Pacific salmon species, the levels varied considerably among populations, with those populations that feed in close proximity to land-based sources of contaminants having higher concentrations. The highest PCB and PBDE concentrations were observed in fish from Puget Sound (53 and 22.5 ng/g ww) and the Harrison River (47 and 17.7 ng/g ww), a subset of the Fraser River Chinook salmon populations. While feeding in marine waters, these populations are primarily distributed within the urbanized waters of the Salish Sea and along the west coast of Vancouver Island (DFO 1999, Weitkamp 2010), with substantial proportions of the populations residing in the Salish Sea rather than migrating to the Pacific Ocean (O'Neill and West 2009, Chamberlin et al. 2011). Interestingly, other populations of Chinook salmon—originating from the developed Fraser River, but with a more northern distribution in the coastal waters of British Columbia and Alaska (DFO 1999)—had much lower PCB and PBDE concentrations (10 and 1.67 ng/g ww). Intermediate levels of PCBs (averages = 22 and 14 ng/g ww) and PBDEs (averages = 3.53 and 2.56 ng/g ww) were measured in California and Oregon populations. Chinook salmon originating from California generally distribute northward in the coastal waters of California, whereas those originating from Oregon disperse both north, to the coastal waters of Washington and the west coast of Vancouver Island, and south, off the coast of California (Weitkamp 2010). Chinook salmon from the Central Valley of California have an adult marine distribution thought to be on the continental shelf. These Chinook salmon have higher ΣDDTs/ΣPCBs ratios than Chinook salmon from the Fraser River and Puget Sound,

which have lower ΣDDTs/ΣPCBs ratios (O'Neill et al. 2006). The higher DDT concentrations that were measured in Chinook salmon originating from California (O'Neill et al. 2006) likely reflect the historically high inputs of DDTs there (Eganhouse et al. 2000). The Alaskan Chinook salmon populations distributed mostly along the remote waters of Alaska (Weitkamp 2010) had the lowest average PCB and PBDE levels (7.7 and 0.67 ng/g ww).

Although elevated POPs in Pacific salmon are primarily driven by their marine distribution and general proximity to land-based sources of contaminants, their high lipid content and trophic status may also contribute to their contaminant concentrations. Lipophilic compounds like PCBs, PBDEs, and DDTs readily accumulate in adult Pacific salmon because of their relatively high fat content. Species and populations differ in their fat content, depending on their diet in salt water and the migratory pattern they undertake (Brett 1995, Quinn 2005), but overall, Chinook salmon and sockeye salmon have higher fat content (O'Neill et al. 2014). Assuming uniform environmental exposures to POPs, adult Chinook salmon and sockeye salmon returning to their natal streams would generally have higher associated POPs than coho salmon, pink salmon, and chum salmon because of their higher fat content. Such comparisons of POP levels among species and populations of Pacific salmon are best made with whole body measurements of POPs, which are less affected by changes in the lipid content of maturing salmon. The lipid content in the muscle tissue of adult salmon in marine waters decreases rapidly as they approach fresh water and reproductive maturity (Brett 1995, Ewald et al. 1998, Hendry and Berg 1999). During this reproductive phase, POPs are not metabolized with the fat, but rather are mobilized and redistributed to fattier tissues such as the gonads (Ewald et al. 1998, Kelly et al. 2007, Veldhoen et al. 2010). Although the POPs are redistributed into fattier tissues, they are not transformed or eliminated (deBruyn et al. 2004), such that whole body POP concentrations in Pacific salmon generally do not decline with reductions in fat content during maturation.

Based on diet alone, POP levels would be highest for Chinook salmon, then coho salmon, followed by sockeye salmon, pink salmon, and chum salmon. Pacific salmon are trophic generalists that consume a mixture of fish and invertebrates while in marine waters. However, the proportion of fish in the diet is greatest for Chinook salmon, followed by coho salmon, then pink salmon and sockeye salmon, and least among chum salmon (Fresh et al. 1981, Peterson et al. 1982, Beacham 1986, Higgs et al. 1995), resulting in a higher trophic level for Chinook salmon (reviewed by Johnson and Schindler 2008) and potentially greater contaminant exposure.

The interaction of lipid content and trophic status on POP accumulation is apparent when comparing Alaskan populations of Pacific salmon that generally are distributed in marine waters distant from land-based sources of contaminants. Average PCB and DDT levels are generally higher for Chinook salmon and sockeye salmon (PCB averages = 7.7 and 14.4 ng/g ww, and DDT averages = 13.0 and 10.4 ng/g ww, respectively) than for coho salmon, pink salmon, and chum salmon (PCB averages = 2.9, 2.2, and 2.7 ng/g ww, and DDT averages = 1.5, 1.2, and 1.9 ng/g ww, respectively). Similarly, average PBDE levels in Alaskan Chinook salmon are considerably higher than for other Alaskan salmon species.

Table 2. Percent lipid and POP concentrations (ng/g wet weight) of adult and subadult Pacific salmon sampled in terminal areas. Terminal areas include coastal marine waters and river mouths through which fish migrate en route to their natal streams. NR = not reported.

Species	Region	Subregion	Population	*n*	Tissue Analyzed	Lipids (%)	PCBs	PBDEs	DDTs	Reference(s)
Chinook salmon	Alaska	unknown	unknown	2	muscle, no skin	NR	5.6	0.95	NR	4
	Alaska	Aleutian Islands	unknown	3	muscle, skin	7.6	5.0	0.71	22	14, 15[a]
	Alaska	SE Alaska/Gulf of Alaska/Bering Sea	unknown	35	muscle, no skin	9.7	11	0.53	7.1	21
	Alaska	SE Alaska	unknown	3	muscle, skin	NR	8.0	0.50	NR	5[a], 6[a]
	Alaska	South Central	River	10	muscle, no skin	NR	9.1	NR	9.8	13
		Alaskan Chinook salmon average				**8.7**	**7.7**	**0.67**	**13.0**	
	British Columbia	BC North Coast	Skeena	30	whole body	NR	7.3	0.08	7.3	11
	British Columbia	Fraser River	Thompson	6	muscle, no skin	10	9.1	NR	1.5	1
	British Columbia	Fraser River		13	whole body	NR	9.4	0.80	6.6	11
	British Columbia	Fraser River	Thompson	7	muscle, no skin	12	8.6	1.54	7.7	17[b]
	British Columbia	Fraser River	Shuswap	2	muscle, no skin	3.0	9.8	NR	5.5	17[b]
	British Columbia	Fraser River	Harrison	6	muscle, no skin	5.4	47	17.7	4.3	1
		Fraser River Chinook salmon average (excluding Harrison)				**8.3**	**10**	**1.67**	**5.7**	
		British Columbia Chinook salmon average				**7.6**	**15**	**4.87**	**5.5**	
	Washington	Puget Sound	Nooksack River	28	muscle, no skin	3.5	37	NR	NR	12
	Washington	Puget Sound	Skagit River	29	muscle, no skin	4.8	40	NR	NR	12
	Washington	Puget Sound	Duwamish River	65	muscle, no skin	7.3	56	NR	NR	12
	Washington	Puget Sound	Nisqually River	20	muscle, no skin	3.8	41	NR	NR	12
	Washington	Puget Sound	Deschutes River	34	muscle, no skin	1.7	59	NR	NR	12
	Washington	Puget Sound	Puget Sound mixed	28	muscle, no skin	4.8	76	NR	NR	12
	Washington	Puget Sound	Duwamish River	3	whole body	6.4	35	6.43	18.3	1
	Washington	Puget Sound	Deschutes River	4	whole body	4.3	56	NR	NR	1
	Washington	Puget Sound	Deschutes River	10	muscle, no skin	1.0	49	NR	NR	8
	Washington	Puget Sound	Issaquah Creek	10	muscle, no skin	0.6	49	NR	NR	8
	Washington	Puget Sound	Puget Sound mixed	36	whole body	NR	43	18.9	29.1	11

[a] Value estimated from figure.
[b] Value estimated from reported lipid weight.

Table 2 continued. Percent lipid and POP concentrations (ng/g wet weight) of adult and subadult Pacific salmon sampled in terminal areas.
Terminal areas include coastal marine waters and river mouths through which fish migrate en route to their natal streams. NR = not reported.

Species	Region	Subregion	Population	*n*	Tissue Analyzed	Lipids (%)	PCBs	PBDEs	DDTs	Reference(s)
Chinook salmon	Washington	Puget Sound	Puget Sound mixed	34	whole body	NR	91	42.2	16.4	11
	Washington	WA Coast	Makah	10	muscle, no skin	1.5	19	NR	NR	8
	Washington	WA Coast	Quinault	10	muscle, no skin	1.8	16	NR	NR	8
		Puget Sound Chinook salmon average				**3.8**	**53**	**22.5**	**21.3**	
		Washington Coast Chinook salmon average				**1.7**	**17**	**NR**	**NR**	
		Washington Chinook salmon average				**3.5**	**48**	**22.5**	**21.3**	
	Oregon	unknown	unknown	3	muscle, skin	NR	10	2.10	NR	5[a], 6[a]
	Oregon	Columbia River	unknown fall	17	whole body	NR	18	3.69	19.9	11
	Oregon	Columbia River	unknown spring	20	whole body	NR	33	9.77	34.8	11
	Oregon	Columbia River	mixed fall Chinook	15	muscle, skin	7.0	37	NR	21.0	18
	Oregon	Columbia River	mixed spring Chinook	24	muscle, skin	9.0	38	NR	22.0	18
	Oregon	Columbia River	fall Chinook	4	whole body	9.4	15	2.30	NR	16
	Oregon	Columbia River	Clackamas River	3	muscle, skin	8.8	13	1.80	NR	16
	Oregon	Columbia River	Clackamas River	3	muscle, no skin	6.1	10	1.50	NR	16
		Oregon Chinook salmon average				**8.1**	**22**	**3.53**	**24.4**	
	California	Sacramento/ San Joaquin	unknown	29	whole body	NR	14	2.56	33.6	11
		Chinook salmon average				**5.6**	**29**	**6.22**	**15.7**	
Sockeye salmon	Alaska	unknown	Alaska	2	muscle, no skin	NR	3.6	0.21	NR	4
	Alaska	Aleutian Islands	unknown	13	muscle, no skin	5.8	130	NR	6.9	3
	Alaska	Kodiak	unknown	3	muscle, skin	NR	5.0	0.10	NR	5[a], 6[a]
	Alaska	Gulf of Alaska/ Bering Sea	unknown	24	muscle, no skin	8.2	13	0.22	12.0	21

[a] Value estimated from figure.

Table 2 continued. Percent lipid and POP concentrations (ng/g wet weight) of adult and subadult Pacific salmon sampled in terminal areas.
Terminal areas include coastal marine waters and river mouths through which fish migrate en route to their natal streams. NR = not reported.

Species	Region	Subregion	Population	*n*	Tissue Analyzed	Lipids (%)	PCBs	PBDEs	DDTs	Reference(s)
Sockeye salmon	Alaska	Gulf of Alaska/ Bering Sea	Copper River	97	muscle, no skin	5.5	37	NR	12.2	19[b]
	Alaska	SE Alaska	unknown	3	muscle, skin	NR	13.3	0.10	NR	5[a], 6[a]
	Alaskan sockeye salmon average					**6.5**	**14.4[c]**	**0.16**	**10.4**	
	British Columbia	unknown	unknown	3	muscle, skin	NR	8.0	0.10	NR	5[a], 6[a]
	British Columbia	Fraser River	Early Stuart	3	soma[d]	16	13	NR	NR	7[b]
	British Columbia	Fraser River	Early Stuart	5	muscle, no skin	4.0	3.9	NR	NR	7[b]
	British Columbia	Fraser River	Early Stuart	6	muscle, no skin	5.0	6.9	NR	NR	7[b]
	British Columbia	Fraser River	Adams	5	muscle, no skin	8.8	7.7	NR	6.6	17[b]
	British Columbia	Fraser River	Weaver Creek	3	muscle, no skin	1.4	6.8	NR	NR	7[b]
	British Columbia	Fraser River	Weaver Creek	2	muscle, no skin	1.1	3.6	NR	NR	7[b]
	British Columbia	Fraser River	Weaver Creek	2	muscle, no skin	1.5	5.3	NR	NR	7[b]
	British Columbia	Fraser River	Weaver Creek	1	muscle, no skin	1.1	4.0	NR	NR	7[b]
	British Columbia	Fraser River	Weaver	8	muscle, no skin	3.9	6.8	NR	5.4	17[b]
	British Columbia	West Coast VI	Great Central Lake	6	muscle	6.1	1.7	NR	NR	7[b]
	British Columbia	West Coast VI	Great Central Lake	3	muscle	6.6	1.6	NR	NR	2[b]
	British Columbia	West Coast VI	Great Central Lake	2	muscle	1.0	1.5	NR	NR	2[b]
	British Columbia	West Coast VI	Great Central Lake	3	muscle	1.0	2.4	NR	NR	2[b]
	British Columbia sockeye salmon average					**4.4**	**5.2**	**0.10**	**6.00**	
	Sockeye salmon average					**4.8**	**7.6[c]**	**0.15**	**8.6**	
Coho salmon	Alaska	unknown	unknown	2	muscle, no skin	NR	1.6	0.32	NR	4
	Alaska	Kodiak	unknown	3	muscle, skin	NR	4.0	0.10	NR	5[a], 6[a]
	Alaska	SE Alaska/ Gulf of Alaska	unknown	14	muscle, no skin	2.9	2.0	0.19	1.5	21
	Alaska	SE Alaska	unknown	3	muscle, skin	NR	4.0	0.10	NR	5a, 6a
	Alaskan coho salmon average					**2.9**	**2.9**	**0.18**	**1.5**	

[a] Value estimated from figure.
[b] Value estimated from reported lipid weight.
[c] Value excluded as an outlier.
[d] Whole body minus gonads.

Table 2 continued. Percent lipid and POP concentrations (ng/g wet weight) of adult and subadult Pacific salmon sampled in terminal areas. Terminal areas include coastal marine waters and river mouths through which fish migrate en route to their natal streams. NR = not reported.

Species	Region	Subregion	Population	*n*	Tissue Analyzed	Lipids (%)	PCBs	PBDEs	DDTs	Reference(s)
Coho salmon	British Columbia	unknown	unknown	3	muscle, skin	NR	6.0	0.30	NR	5[a], 6[a]
	Washington	Puget Sound	unknown	32	muscle, no skin	3.1	35	NR	NR	10
	Washington	Puget Sound	Puget Sound mixed	125	muscle, no skin	3.1	27	NR	NR	10
	Washington	Puget Sound	Puget Sound mixed	266	muscle, no skin	3.3	NR	NR	11.7	20
	Washington coho salmon average					**3.2**	**31**	**NR**	**11.7**	
	Oregon	Columbia River	Umatilla River	3	muscle, skin	2.5	35	NR	41.0	18
	Coho salmon average					**3.0**	**14**	**0.20**	**18.1**	
Pink salmon	Alaska	Kodiak	unknown	3	muscle, skin	NR	3.0	0.10	NR	5[a], 6[a]
	Alaska	northern Alaska	unknown	7	canned	6.3	2.6	NR	1.8	22
	Alaska	SE Alaska/GOA	unknown	12	muscle, no skin	3.5	1.3	0.22	0.6	21
	Alaska	SE Alaska	unknown	3	muscle, skin	NR	2.0	0.10	NR	5[a], 6[a]
	Alaskan pink salmon average					**4.9**	**2.2**	**0.14**	**1.2**	
	British Columbia	unknown	unknown	3	muscle, skin	NR	3.0	0.30	NR	5[a], 6[a]
	Pink salmon average					**4.9**	**2.4**	**0.18**	**1.2**	
Chum salmon	Alaska	Kodiak	unknown	3	muscle, skin	NR	2.0	0.10	NR	5[a], 6[a]
	Alaska	SE Alaska	unknown	3	muscle, skin	NR	3.0	0.10	NR	5[a], 6[a]
	Alaska	Bering Sea	unknown	18	muscle, no skin	4.8	3.2	0.16	1.9	21
	Alaskan chum salmon average					**4.8**	**2.7**	**0.12**	**1.9**	
	British Columbia	unknown	unknown	3	muscle, skin	NR	2.0	0.20	NR	5[a], 6[a]
	Chum salmon average					**4.8**	**2.6**	**0.14**	**1.9**	

References: 1) Cullon et al. (2009), 2) deBruyn et al. (2004), 3) Hardell et al. (2010), 4) Hayward et al. (2007), 5) Hites et al. (2004a), 6) Hites et al. (2004b), 7) Kelly et al. (2007), 8) Missildine et al. (2005), 9) Montory et al. (2010), 10) O'Neill et al. (1998), 11) O'Neill et al. (2006), 12) O'Neill and West (2009), 13) Rice and Moles (2006), 14) Shaw et al. (2008), 15) Shaw et al. (2006), 16) Stone (2006), 17) Veldhoen et al. (2010), 18) EPA (2002), 19) Ewald et al. (1998), 20) West et al. (2001), 21) ADEC (2011), 22) O'Hara et al. (2005)

[a] Value estimated from figure.

Table 3. Concentrations (±) of summed PCBs, PBDEs, and DDTs (lipid weight, ng/g) measured in blubber collected from killer whales and other cetaceans from the North Pacific. NR = not recorded; LOQ = limit of quantification.

Species	Age, Sex-Class, and/or Ecotype	Collection Region	Collection Year(s)	*n*	Lipid Weight (ng/g)			Ref.
					ΣPCBs	ΣPBDEs	ΣDDTs	
Bowhead whale[a] (*Balaena mysticetus*)	NR	Beaufort–Chukchi Sea, Alaska	1999–2000	25	540 ± 45	NR	440 ± 40	1
Fin whale[b] (*Balaenoptera physalus*)	Male (unknown age)	Gulf of California	2004–2005	9	200 ± 30	NR	1,400 ± 220	2
Fin whale[b]	Female (unknown age)	Gulf of California	2004–2005	12	130 ± 25	NR	950 ± 200	2
Gray whale[a] (*Eschrichtius robustus*)	Juvenile	Russia	1994	17	1400 ± 130	NR	330 ± 53	3
Gray whale[b]	Unknown	Neah Bay, WA	1996–1998	38	220 ± 42	NR	130 ± 26	4
Humpback whale[b]	Male (unknown age)	Southern California	2004	5	800 ± 390	180 ± 56	4,900 ± 2,600	5
Humpback whale[b]	Male (unknown age)	Northern California	2004	5	140 ± 60	55 ± 26	760 ± 230	5
Humpback whale[b]	Male (unknown age)	Washington State	2004	10	560 ± 140	100 ± 29	1,400 ± 350	5
Humpback whale[b]	Male (unknown age)	Southeast Alaska	2003–2004	10	430 ± 97	22 ± 6	830 ± 130	5
Humpback whale[b]	Male (unknown age)	North Gulf of Alaska	2004	8	110 ± 86	<LOQ	200 ± 120	5
Humpback whale[b]	Male (unknown age)	West Gulf of Alaska	2004	9	390 ± 180	8	610 ± 320	5
Humpback whale[b]	Male (unknown age)	East Aleutian Islands	2004	10	200 ± 60	<LOQ	320 ± 73	5
Humpback whale[b]	Male (unknown age)	Bering Sea	2004	10	160 ± 24	<LOQ	170 ± 39	5
Beluga[a] (*Delphinapterus leucas*)	Mix	Pt. Lay, AK	1999–2000	20	3300 ± 310	NR	2,000 ± 230	1
Beluga[a]	Mix	Cook Inlet, AK	2001–2002	4	1,700 ± 1,100	NR	2,300 ± 1600	6
False killer whale[b] (*Pseudorca crassidens*)	Juvenile/Sub-adult	Main Hawaiian Islands	2008	2	19,000 ± 7,100	2700 ± 350	20,000 ± 4900	7
False killer whale[b]	Adult male	Main Hawaiian Islands	2008	2	33,000 ± 0	1200 ± 580	63,000 ± 28,000	7

[a] Blubber collected from subsistence-harvested animals.
[b] Blubber collected from biopsied animals.

Table 3 continued. Concentrations (±) of summed PCBs, PBDEs, and DDTs (lipid weight, ng/g) measured in blubber collected from killer whales and other cetaceans from the North Pacific. NR = not recorded; LOQ = limit of quantification.

Species	Age, Sex-Class, and/or Ecotype	Collection Region	Collection Year(s)	*n*	Lipid Weight (ng/g) ΣPCBs	ΣPBDEs	ΣDDTs	Ref.
False killer whale[b]	Adult female	Main Hawaiian Islands	2008	5	3,500 ± 4,200	420 ± 720	3,000 ± 3,000	7
Killer whale[c]	Residents, Transients	Puget Sound/British Columbia	1986–1989	6	22,000	NR	32,000	8
Killer whale[b]	Adult male (Northern Residents)	Puget Sound/British Columbia	1993–1996	8	37,000 ± 6,100	200 ± 120 (n = 13)	NR	9, 10
Killer whale[b]	Adult female (Northern Residents)	Puget Sound/British Columbia	1993–1996	9	9,300 ± 2,800	420 ± 680 (n = 8)	NR	9, 10
Killer whale[b]	Adult male (Southern Residents)	Puget Sound/British Columbia	1993–1996	4	150,000 ± 33,000	940 ± 580 (n = 5)	NR	9, 10
Killer whale[b]	Adult female (Southern Residents)	Puget Sound/British Columbia	1993–1996	2	55,000 ± 19,000	NR	NR	9, 10
Killer whale[b]	Male (transients)	Puget Sound/British Columbia	1993–1996	5	250,000 ± 55,000	1,000 ± 610 (n = 6)	NR	9, 10
Killer whale[b]	Female (transients)	Puget Sound/British Columbia	1993–1996	5	59,000 ± 21,000	890 ± 710 (n = 6)	NR	9, 10
Killer whale[b]	Juvenile/Sub-adult (Southern Residents)	Puget Sound/British Columbia	2004–2007	4	44,000 ± 12,000	14,000 ± 2,400	51,000 ± 34,000	11, 12
Killer whale[b]	Adult male (Southern Residents)	Puget Sound/British Columbia	2004–2007	10	56,000 ± 46,000	3,600 ± 1,600	82,000 ± 38,000	11, 12
Killer whale[b]	Adult female (Southern Residents)	Puget Sound/British Columbia	2004–2007	7	37,000 ± 42,000	3,700 ± 2,800	30,000 ± 34,000	11, 12
Killer whale[b,c]	Juvenile/Sub-adult (Southern Residents)	Puget Sound/British Columbia	2008–2013	21	39,000 ± 19,000	5,300 ± 2,600	68,000 ± 50,000	13
Killer whale[b]	Adult male (Southern Residents)	Puget Sound/British Columbia	2008–2013	4	40,000 ± 28,000	4,700 ± 3,000		13

[b] Blubber collected from biopsied animals.
[c] Blubber collected from stranded animals.

Table 3 continued. Concentrations (±) of summed PCBs, PBDEs, and DDTs (lipid weight, ng/g) measured in blubber collected from killer whales and other cetaceans from the North Pacific. NR = not recorded; LOQ = limit of quantification.

Species	Age, Sex-Class, and/or Ecotype	Collection Region	Collection Year(s)	*n*	Lipid Weight (ng/g) ΣPCBs	ΣPBDEs	ΣDDTs	Ref.
Killer whale[b]	Adult female (Southern Residents)	Puget Sound/British Columbia	2008–2013	22	30,000 ± 31,000	3,700 ± 3,200	41,000 ± 54,000	13
Killer whale[c]	Adult male	Japan (Shiretoko Peninsula)	2005	1	57,000	270	220,000	14
Killer whale[c]	Calf	Japan (Shiretoko Peninsula)	2005	3	50,000 ± 13,000	500 ± 36	140,000 ± 55,000	14
Killer whale[c]	Adult female	Japan (Shiretoko Peninsula)	2005	5	31,000 ± 10,000	260 ± 72	66,000 ± 34,000	14
Killer whale[b]	Mix (transients)	Kenai Fjords/Prince William Sound, AK	1994–1999	64	230,000 ± 130,000	NR	320,000 ± 210,000	15
Killer whale[b]	Mix (residents)	Kenai Fjords/Prince William Sound, AK	1994–1999	13	14,000 ± 13,000	NR	13,000 ± 14,000	15
Killer whale[b]	Adult male (residents)	Central Aleutian Islands, AK	2003–2004	3	15,000 ± 1,100	36 ± 32	26,000 ± 1,200	16
Killer whale[b]	Adult male (residents)	Eastern Aleutian Islands, AK	2003–2004	20	16,000 ± 4,900	50 ± 29	27,000 ± 12,000	16
Killer whale[b]	Adult male (residents)	Gulf of Alaska	2003–2004	17	9,300 ± 5,200	120 ± 89	13,000 ± 7,800	16
Killer whale[b]	Adult male (transients)	Eastern Aleutian Islands, AK	2003–2004	15	120,000 ± 49,000	790 ± 590	200,000 ± 110,000	16
Killer whale[b]	Adult male (transients)	West Coast (California)	2000–2001	4	630,000 ± 190,000	12,600	3,700,000 ± 910,000	16
Killer whale[b]	Adult male (offshores)	Alaska	2003–2004	4	110,000 ± 22,000	3,300 ± 940	420,000 ± 100,000	16

References: 1) Hoekstra et al. (2003), 2) Niño-Torres et al. (2010), 3) Tilbury et al. (2002), 4) Krahn et al. (2001), 5) Elfes et al. (2010), 6) Krahn et al. (2004), 7) Ylitalo et al. (2009), 8) Jarman et al. (1996), 9) Ross et al. (2000), 10) Rayne et al. (2004), 11) Krahn et al. (2007a), 12) Krahn et al. (2009), 13) NWFSC unpubl. data, 14) Kajiwara et al. (2006), 15) Ylitalo et al. (2001), 16) Krahn et al. (2007b)

[b] Blubber collected from biopsied animals.
[c] Blubber collected from stranded animals.

Factors Influencing Bioaccumulation in Whales

Several chemical and biological factors are known to influence the bioaccumulation of lipophilic contaminants and the total body burdens in marine mammals. There can be large differences in concentrations among individuals within a population and between populations. Several studies have reported on the levels of POPs in killer whales and other marine mammals from the North Pacific (Table 3). These data come from both stranded animals (e.g., Calambokidis et al. 1984, Jarman et al. 1996) and blubber biopsy samples of wild-ranging animals (details on biopsy techniques in Ylitalo et al. 2001, Ikonomou et al. 2007, Noren and Mocklin 2012). Relative to other marine mammal species, killer whales accumulate high levels of several POPs, predominantly ΣPCBs and p,p'-DDE, a major metabolite of DDT (Table 3). Below we review the primary factors influencing the bioaccumulation of POPs in whales.

Influence of Geographic Distribution and Diet on POP Accumulation

As mentioned above, adult marine mammals receive the majority of POPs from their prey (e.g., de Swart et al. 1994); therefore, diet and trophic position are primary factors that influence accumulation. The effect of diet is significant for POPs that biomagnify up the food chain, and therefore top predators generally have higher levels than lower trophic-level species. For example, transient killer whales eat marine mammals, which are relatively high on the food chain. These transient killer whales have significantly higher ΣPCBs and ΣDDTs than resident killer whale communities that eat fish (Ross et al. 2000, Ylitalo et al. 2001), revealing the influence of diet and trophic position on contaminant concentrations. Diet and trophic position are thought to explain most of the variation of POP levels among species, and may contribute to the variation among populations of the same species or within a population if diet is age- or sex-specific (Aguilar et al. 1999).

POPs in marine mammals can also vary based on geographic proximity to contaminated marine environments. Killer whales in Washington and British Columbia have notably higher POP levels than other North Pacific killer whales at similar trophic levels (Table 3), likely indicating a higher regional input of these contaminants. Krahn et al. (2008) compared patterns of POPs in Antarctic Type C killer whales to Alaska offshores, Alaska residents, Alaska and West Coast transients, and Eastern Tropical Pacific (ETP) killer whales. With the exception of hexachlorobenzene (HCB), Antarctic Type C killer whales had the lowest measured POPs of all the studied killer whale communities. These lower levels of POPs in Antarctic Type C killer whales were not unexpected considering the low potential of regional input.

POP levels in the blubber of humpback whales (*Megaptera novaeangliae*) from the eastern North Pacific were also recently measured to better understand the geographic distribution of contaminants (Elfes et al. 2010). Humpback whales serve as good bioindicators of contamination because they are seasonal feeders with strong site fidelity to feeding regions (Elfes et al. 2010). The researchers found that humpback whales that feed off California and Washington had higher

concentrations of ΣPCBs, ΣPBDEs, and ΣDDTs than whales feeding in higher-latitude regions. ΣPBDEs were not detected in whales sampled in the northern Gulf of Alaska, eastern Aleutian Islands, or Bering Sea. ΣDDTs were exceptionally high in humpbacks that feed off California. Furthermore, whales that feed in southern California had six times the levels of ΣDDTs as whales that feed in northern California (Elfes et al. 2010).

Dynamics of POP Transfer from Mother to Offspring

Marine mammals not only receive contaminants from the prey they consume; young marine mammals also receive contaminants from their mother, offloaded via transplacental transfer during gestation and via milk during lactation (e.g., Duinker and Hillebrand 1979, Donkin et al. 1981, Tanabe et al. 1982, Addison and Brodie 1987, Aguilar and Borrell 1994, Borrell et al. 1995, Ridgway and Reddy 1995, Lee et al. 1996, Pomeroy et al. 1996, Ylitalo et al. 2001, Debier et al. 2003, Desforges et al. 2012). Indeed, the total contaminant load accumulated in a cetacean calf prior to consuming prey depends solely on the mother's contaminant load. In addition, cetacean contaminant burdens are directly related to birth order; first-born young receive greater contaminant loads from their mothers than subsequent offspring (Ylitalo et al. 2001).

Transfer rates of specific contaminants within each class tend to depend on molecular weight and degree of lipophilicity. Specifically, PCBs with higher molecular weights do not appear to transfer across the placenta (Salata et al. 1995) and are not easily mobilized from mother to offspring during gestation (Tanabe et al. 1981, Tanabe et al. 1982) or during lactation (Park et al. 2010). In general, during gestation and lactation in both pinnipeds and delphinids, the most easily transferred organochlorines are HCH and HCB, followed by DDTs and then PCBs (Fukushima and Kawai 1981, Tanabe et al. 1982, Addison and Brodie 1987, Aguilar 1987, Borrell et al. 1995), as shown in Table 4.

Several biological factors also influence transfer rates during gestation and lactation. For example, Tanabe et al. (1982) proposed that transplacental transfer rates in delphinids depend on the ratio of the body weight of the fetus to that of the pregnant female. Although more data are needed to support this, the trend does seem to exist. Tanabe et al. (1982) estimated that a 25.5-year-old female striped dolphin (*Stenella coeruleoalba*) transferred 4.0% of her body burden of PCBs and 4.7% of DDTs to her fetus, whose mass was 6.1% that of the female, during gestation. Borrell et al. (1995) estimated that the average transfer rate during gestation in long-finned pilot whales (*Globicephala melas*) for PCBs (~7%) and DDTs (~8%) was nearly double that reported by Tanabe et al. (1982) for striped dolphins. The ratio of fetus weight to mother weight (~9%) in long-finned pilot whales is also higher than that for striped dolphins (Borrell et al. 1995). Furthermore, a gestational transfer rate of 15% for organochlorine compounds was estimated for harbor porpoise (*Phocoena phocoena*), whose calves are approximately 17% the mass of females (Duinker and Hillebrand 1979). Offloaded amounts of contaminants also vary by individual female and are related to age and reproductive history. Specifically, transfer rates tend to decrease with a mother's age, and are consequently much higher in primiparous females than in those that have already given birth (Aguilar and Borrell 1994, Borrell et al. 1995). For example, Fukushima and Kawai

Table 4. Contaminant transfer from delphinid and phocoenid females to calves during gestation and/or lactation.

Contaminant	Species	% Offload (Gestation)	% Offload (Lactation)	% Offload (Total)	Reference
ΣPCB	Melon-headed whale	3.5%, 3.6%	——	89%	Kajiwara et al. (2008) (n = 2)
	Beluga	11.4%			Desforges et al. (2012)
	Bottlenose dolphin	3.7%	——	—	Salata et al. (1995)
	Long-finned pilot whale	(9-yr-old) 9.73% (13-yr-old) 6.09% (30-yr-old) 4.14%	(9-yr-old) 99.9% (13-yr-old) 95.5% (30-yr-old) 92.5%	—	Borrell et al. (1995)
	Striped dolphin	(25.5-yr-old) 4.0%	—	—	Tanabe et al. (1982)
		— (25.5-yr-old) 4.0%	(17.5-yr-old) 92.6% (25.5-yr-old) 91.1%	—	Tanabe et al. (1981) (values may be overestimated)
		3.8%	88%	—	Fukushima and Kawai (1981) (assumed maximum rates)
ΣPBDE	Beluga	11.1%	—	—	Desforges et al. (2012)
ΣDDT	Melon-headed whale	2.6%, 3.5%	—	85%	Kajiwara et al. (2008)
	Bottlenose dolphin	5.1%	—	—	Salata et al. (1995)
	Long-finned pilot whale	(9-yr-old) 9.58% (13-yr-old) 7.85% (30-yr-old) 6.95%	(9-yr-old) 67.6% (13-yr-old) 80.7% (30-yr-old) 100%	—	Borrell et al. (1995)
	Striped dolphin	(ΣDDT) 4.7% (p,p'-DDE) 5.4% (p,p'-DDD) 6.6% (p,p'-DDT) 3.0%	— — — —	— — — —	Tanabe et al. (1982) (25.5-yr-old female)

Table 4 continued. Contaminant transfer from delphinid and phocoenid females to calves during gestation and/or lactation.

Contaminant	Species	% Offload (Gestation)	% Offload (Lactation)	% Offload (Total)	Reference
ΣDDT	Striped dolphin	—	(17.5-yr-old) 95.0%	—	Tanabe et al. (1981)
		(2.5-yr-old) 4.7%	(25.5-yr-old) 94.6%		(values may be overestimated)
		4.2%	91%	—	Fukushima and Kawai (1981) (assumed maximum rates)
TCPMOH	Melon-headed whale	3.3%, 5.3%	—	84%	(n = 2)
TCPMe		0.55%, 1.2%	—	41%	
HP-epox		2.7%, 3.5%	—	83%	
ΣCHL		5.0%, 5.8%	—	89%	
HCB		3.7%, 5.3%	—	76%	
	Striped dolphin	(25.5-yr-old) 9.4%	—	—	Tanabe et al. (1982)
	Striped dolphin	—	(17.5-yr-old) 98.1%	—	Tanabe et al. (1981)
		(25.5-yr-old) 9.4%	(25.5-yr-old) 98.0%		(values may be overestimated)
ΣHCH	Melon-headed whale	5.6%, 6.0%	—	82%	Kajiwara et al. (2008) (n = 2)
ΣHCH	Striped dolphin	8.9%	—	—	Tanabe et al. (1982)
α-HCH		7.0%	—	—	(25.5-yr-old female)
β-HCH		9.7%	—	—	
γ-HCH		6.5%	—	—	
ΣBHC	Striped dolphin	—	(17.5-yr-old) 94.9%	—	Tanabe et al. (1981)
		(25.5-yr-old) 8.9%	(25.5-yr-old) 93.5%		(values may be overestimated)
		6.3%	72%	—	Fukushima and Kawai (1981) (assumed maximum rates)
Nonspecific organochlorine compounds	Harbor porpoise	15%	—	—	Duinker and Hillebrand (1979)

(1981) suggested that first-born dolphin calves receive a fourfold higher initial burden of PCBs and DDTs than subsequent calves, and that approximately 90% of this load is transferred through lactation. Cockcroft et al. (1989) also reported that first-born bottlenose dolphin (*Tursiops truncatus*) calves receive the majority of their mothers' contaminant loads during lactation.

Regardless of maternal age (and presumably reproductive history), transfer rates during lactation are always much greater than during gestation (Fukushima and Kawai 1981, Tanabe et al. 1981, Tanabe et al. 1982, Borrell et al. 1995). Thus, lactation is the major elimination route of contaminants for female cetaceans. Currently, the quantity of contaminants typically offloaded from mother to calf in killer whales is unknown. However, PCBs and DDTs offload data are available for other delphinids. The ranges of transfer rates of PCBs and DDTs during gestation are 3.7–9.73% and 4.2–9.58%, respectively, of a female's total body load. During lactation, the ranges of transfer rates of PCBs and DDTs are 88–99.9% and 67.6–100%, respectively, of a female's total body load (Table 4).

Given the higher transfer rates during lactation, a large quantity of contaminants could be delivered to a suckling calf in a short amount of time. For example, Cockcroft et al. (1989) calculated that approximately 4% of a female bottlenose dolphin's total body burden can be transferred to her calf during lactation daily; consequently, the mother's full load would be transferred after approximately seven weeks of lactation. Interestingly, the one study (Aguilar and Borrell 1994) that estimated transfer rates for a mysticete (in this case, fin whales) found much lower total transfer rates for each reproductive cycle (gestation and lactation combined). Depending on the age of the female, transfer rates during each reproductive cycle ranged from 3–14% and 9–27% of a female's total body load for PCBs and DDTs, respectively. The authors attributed the relatively low transfer rates to the shorter lactation duration of fin whales (approximately 7 months) compared to the longer lactation periods (18 months) of striped and bottlenose dolphins (Aguilar and Borrell 1994). PCBs and DDTs transfer rates of phocid seals that have relatively short lactation periods are near the range of those reported for fin whales (Kajiwara et al. 2008). Similar to delphinids, younger female fin whales offloaded a higher percentage of their body burden to their calves compared to older females that had reproduced more than once (Aguilar and Borrell 1994). These examples illustrate the importance of having a good understanding of the duration of lactation, in addition to individual females' reproductive histories, when assessing contaminant transfer rates from female killer whales to their calves.

Less is known about the transfer of PBDEs during gestation and lactation in cetaceans. However, it is reasonable to assume that the transfer rates of PCBs and PBDEs are comparable, because the chemical structures of these two classes of contaminants are similar. Recent studies have shown that maternal transfer of PBDEs during gestation and lactation occurs (Kajiwara et al. 2008, Park et al. 2010, Desforges et al. 2012) and that transfer rates of PCBs and PBDEs are comparable (Table 4). For example, ΣPCB and ΣPBDE transfer patterns over the lactation period in bottlenose dolphins tend to be similar (D. Noren unpubl. data). Estimated placental transfer rates for two female melon-headed whales (*Peponocephala electra*) ranged from 3.5–3.6% and 2.6–3.5% for PCBs and PBDEs, respectively. The estimated transfer rates for gestation and lactation combined were 89% and 85% for PCBs and PBDEs, respectively (Kajiwara et al. 2008). Although transfer

rates for killer whales have not been reported, recent studies have found that cetacean calves and young juveniles have blubber PBDE concentrations that are orders of magnitude greater than those of reproductive females (Krahn et al. 2007a, Haraguchi et al. 2009, Krahn et al. 2009, Ylitalo et al. 2009). Similar to the more highly chlorinated PCBs, the more highly brominated PBDE compounds are less transferable from delphinid and phocoenid females to their fetuses during gestation and to their calves during lactation (Kajiwara et al. 2008, Haraguchi et al. 2009, Park et al. 2010) because of their larger molecular size and higher lipophilicity.

Other Influential Factors Affecting POP Accumulation

Age- and sex-related patterns of POP levels in marine mammals have been well cited in the literature. Persistent pollutants will accumulate in marine mammals when the input or exposure exceeds the ability of the individual to metabolize and excrete them. As a result of this bioaccumulation, concentrations can increase with the individual's age (Ross et al. 2000, Ylitalo et al. 2008). However, this age-specific pattern is different in males and females. As mentioned above, reproductive females are able to offload their contaminants to their offspring via transplacental transfer and lactation. Therefore, reproductive females that have given birth to calves and nursed, or are pregnant, can have significantly lower POP levels than adult males of a similar age. For example, male and female resident killer whales have distinct age-related patterns of ΣPCB and ΣDDT accumulation. In males, these POPs continuously increase with age, allowing them to accumulate relatively high body burdens (Ross et al. 2000, Krahn et al. 2007a). In females, levels decline and remain low during reproductive years, and subsequently increase again in older females, likely reflecting reproductive senescence (Ross et al. 2000).

Not all POP levels follow this general age- or sex-related pattern. For example, ΣPBDE concentrations in male Southern Residents and male and female transients sampled during the 1990s did not differ, nor did these pollutants increase with age (Rayne et al. 2004). The difference in age-related patterns between the legacy PCBs and the PBDEs may be due in part to PBDEs being relatively new in the environment (Rayne et al. 2004, Mongillo et al. 2012). Thus, older individuals were not exposed when they were young and do not have a lifetime of accumulation. The difference may also be due to differences in metabolism and excretion of PBDEs among age classes. Adults may have a higher ability to metabolize these compounds than juveniles do. For example, the highest levels of PBDEs were measured in juveniles, not adults, in both Southern Residents and main Hawaiian Island false killer whales (Krahn et al. 2007a, Krahn et al. 2009, Ylitalo et al. 2009).

Although cetaceans in general have a lower ability to metabolize certain lipophilic compounds than other mammals, killer whales may have some ability to metabolize some compounds (Wolkers et al. 2007). Unlike the ΣPCBs, ΣDDTs, and ΣPBDEs that have been measured at high concentrations in killer whales, other POPs, such as the polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDDs and PCDFs), were low or undetectable in eastern North Pacific killer whales (Ross et al. 2000). Similarly, low POP concentrations of the hexachlorocyclohexanes α-HCH and γ-HCH in the blubber tissues of North Atlantic killer

whales were also reported (McHugh et al. 2007). These patterns of high ΣPCBs and ΣDDTs and low dioxins, furans, and HCHs suggest the ability of these whales to either metabolize or regulate certain congeners, which plays a major role in the accumulation and retention of certain chemicals (Ross et al. 2000, McHugh et al. 2007).

Wolkers et al. (2007) examined congener-specific metabolism and accumulation in killer whales from Norway, as well as the transfer of contaminants from their main prey, herring. They found that the more persistent PCB congeners and DDE can accumulate to some degree in killer whales, reflecting the whales' inability to metabolize these compounds. Additionally, they suggested killer whales may have the ability to metabolize most PBDEs, but despite this, there are a few PBDE congeners that accumulate. Evidence of this is found in congener-specific contaminant profiles from different killer whale populations. For example, congener-specific PCB profiles are similar across killer whale ecotypes, with the more highly chlorinated congeners dominating, especially the ortho-substituted congeners -138 and -153 (Ross et al. 2000). Ylitalo et al. (2001) also found that PCB congeners -138 and -153 dominated in killer whale blubber. More specifically, PCB congeners -52, -101, -118, -138, -153, and -180 accounted for almost half of the ΣPCB concentrations in Southern Resident killer whale blubber (Ross et al. 2000). The more toxic non-ortho-substituted congeners -77, -126, and -169 were not detected in the blubber of killer whales from Kenai Fjords/Prince William Sound, Alaska (Ylitalo et al. 2001). Similar PBDE congener profiles were also observed across killer whale populations. For example, Rayne et al. (2004) sampled transients, Northern Resident killer whales, and Southern Resident killer whales for PBDEs, and although the congener contribution varied among the killer whales, concentrations of PBDEs -47, -99, -100, -153, and -154 were higher than the other PBDE congeners (Rayne et al. 2004), reflecting the killer whales' inability to excrete these persistent congeners.

Body composition and nutritive condition are additional factors that affect the dynamics of POP accumulation in marine mammals. Body size may also influence the accumulation pattern of pollutants; however, the effect is complex (Aguilar et al. 1999). Because POPs are generally lipophilic and accumulate in fatty tissues, species that have more fatty tissue will have a larger capacity for storing these pollutants. Approximately 70–95% of the total contaminant body load in whales is in the lipid-rich blubber (Aguilar et al. 1999, Yordy et al. 2010b). Blubber can serve as an energy store, and its contribution to body mass will depend on nutritive condition. The nutritive condition of an individual will affect both the volume and composition of the fat. When lipids are mobilized (e.g., because of seasonal fluctuations or reproductive status), the POPs can either remain in the blubber and become more concentrated, or become mobilized along with the lipids (Aguilar 1987, Krahn et al. 2002, Debier et al. 2006, Yordy et al. 2010c, Louis et al. 2014). POPs may be selectively mobilized from the blubber when lipids are mobilized, based on their physicochemical properties (Yordy et al. 2010c). Once POPs enter the bloodstream, they may redistribute, leading to elevated tissue concentrations (Yordy et al. 2010c). During the early post-weaning fast in northern elephant seal (*Mirounga angustirostris*) pups, the fatty acids from the inner blubber layer are mobilized into circulation, whereas the rate of mobilization of POPs remains relatively low (Debier et al. 2006, Louis et al. 2014). During this early fasting period, POP concentrations are significantly higher in the inner blubber layer than the outer blubber layer. As the period of fasting progresses, POP concentrations increase in the pups' serum (Debier et al. 2006, Louis et al. 2014). Therefore, the effects of POPs may be compounded by stresses associated with nutritional limitation.

Potential Adverse Health Effects from POP Exposure

Various deleterious biological effects have been associated with exposures to PCBs, PBDEs, and DDTs in humans, laboratory animals, and wildlife. These pollutants have the ability to disrupt the endocrine system, nervous system, and immune system, and also cause cancer. In this section we discuss these adverse health effects from isolated POPs observed in wildlife and laboratory species, with a particular focus on PCBs and PBDEs in detail, and we include brief descriptions of the endocrine, nervous, and immune systems in marine mammals.

Influence of Organic Contaminants on the Endocrine System

Mammals have a sophisticated endocrine system that controls their physiological and metabolic processes. In general, the endocrine system consists of several endocrine glands that produce and regulate hormones, which play essential roles in several aspects of reproduction, neural development, and growth, in the regulation of metabolism, and in controlling heat loss. Some of the hormones produced in endocrine glands are released into the bloodstream, where they can travel to target cells, interact with hormone receptors, and activate a response (e.g., gene activation). The main parts of the endocrine system include the hypothalamus, pituitary gland, thyroid gland, adrenal glands, pineal gland, and the gonads (Figure 4). Below, we provide a general background on thyroid and sex hormones, followed by the health effects on these hormones that have been observed in wildlife and laboratory species as a result of exposure to organic contaminants.

Thyroid Hormones

The hypothalamus, located in the brain, links the nervous system with the endocrine system. The hypothalamus sends a thyroid releasing hormone (TRH) to the pituitary gland. This gland releases thyrotropin (TSH), which stimulates the production of thyroid hormones from the thyroid gland (Figure 4). Thyroid hormones, such as thyroxine (T_4) and triiodothyronine (T_3), are fat-soluble and are transported into circulation bound to the carrier protein complexes transthyretin (TTR) or T_4-binding globulin (TBG). If they are not bound to a carrier protein, they are readily removed from the blood by the kidneys and liver. T_4 is the most abundantly secreted hormone, but only a fraction of the total T_4 enters target cells (i.e., cells that change their gene expression in response to the hormone). Once T_4 is transported to a target cell, it is deiodinated to its biologically active form, T_3, which then binds with nuclear receptors (NRs) that regulate the expression of certain genes (Figure 4). T_3 is then released into circulation and recycled or excreted via the bile.

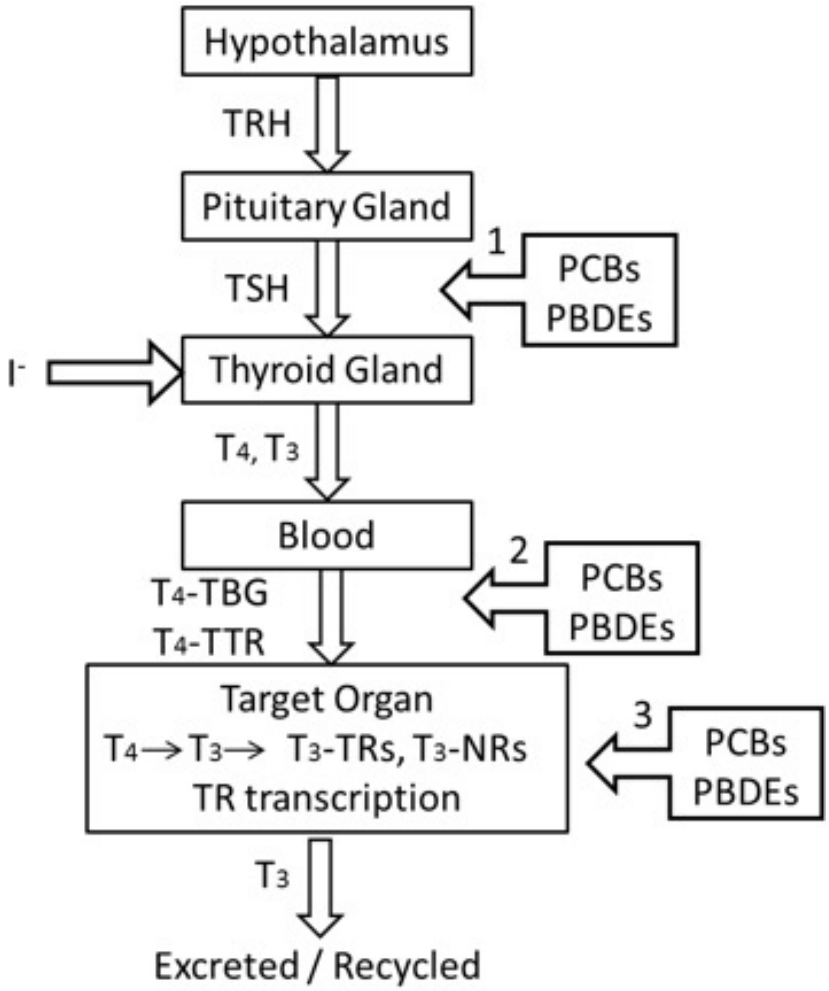

Figure 4. Primary components of the hypothalamic–pituitary–thyroid axis. The hypothalamus releases the thyroid releasing hormone (TRH), a hormone that stimulates the secretion of thyrotropin (TSH). TSH acts on the thyroid gland to stimulate iodide (I^-) uptake, and regulates the synthesis and release of thyroid hormones (T_4 and T_3). T_4 binds to transport proteins (TBG or TTR) and enters into circulation. Once the T_4 reaches a target cell, it is converted to T_3, which binds to thyroid hormone receptors (TRs) and nuclear receptors (NRs) and activates transcription. T_3 is then excreted via bile or recycled. The potential mechanisms of action for PCBs and PBDEs on the hypothalamic–pituitary–thyroid axis include: 1) inhibition of the synthesis of thyroid hormones by interfering with the TSH receptor; 2) binding to the transport protein; and/or 3) binding to the thyroid nuclear receptor and altering gene expression.

In pinnipeds, reported levels of circulating T_4 have ranged from 4 to 190 µg/L, whereas T_3 concentrations are much lower, ranging from 1.04 to 1.76 µg/L (see Gregory and Cyr 2003 and references within). There are several influential factors that affect TH concentrations in pinnipeds that may explain the variability observed. For example, thyroid hormone concentrations decrease with age or maturation in several pinnipeds (see Myers et al. 2006 and references within). Thyroid hormones have also been shown to vary by season in some marine mammals (e.g., harbor seals; Oki and Atkinson 2004), and have been associated in some pinnipeds with the annual molt (see Myers et al. 2006 and references within).

In cetaceans, reported levels of circulating T_4 have ranged from 5 to 190 µg/L, similarly to pinnipeds (Gregory and Cyr 2003). Thyroid hormones in cetaceans can also be influenced by several factors, including age, sex, diet (e.g., goitrogens), and season, and can vary by geographic region. For example, thyroid hormone levels were significantly higher in bottlenose dolphins from the coastal waters of South Carolina compared to those from Florida, which is likely attributed to coping with lower year-round temperatures (Fair et al. 2011). However, age had the most influence on TH concentrations, with decreasing concentrations measured with increasing age (Fair et al. 2011). A seasonal cycle in thyroid activity has also been described in belugas, where TH levels corresponded to the whales' distinct seasonal use of warm river estuaries and cold ocean habitat (St. Aubin and Geraci 1989). In contrast, a seasonal cycle of THs was not apparent in Atlantic bottlenose dolphins, and sex had the most influence on TH concentrations with higher levels found in females (St. Aubin et al. 1996). Southern Resident killer whales that were sampled in late spring/early summer had higher fecal T_3 levels than whales sampled in late fall/early winter (Ayres et al. 2012). The degree of influence these factors have on the endocrine system in marine mammals appears to be complex and species-specific, warranting further research.

Effects on Thyroid Hormones from Organic Contaminant Exposure

In addition to the influential factors described above, several contaminants are considered potential endocrine disruptors and can interfere with hormone signaling or activate signal pathways, acting as agonists or antagonists (i.e., enhancing or inhibiting the effect of hormones). In general, some endocrine disrupting chemicals (EDCs) are structurally similar to T_4 and T_3, and can affect the TH system through several potential mechanisms of action (Figure 4). For example, some EDCs may inhibit the synthesis of THs; modify the metabolism of THs; displace T_4 and bind to TH receptors or transport proteins, reducing the transfer of retinol (vitamin A) and T_4 to target organs; interfere with cellular uptake mechanisms; alter gene expression; and decrease the availability of progesterone (de Boer et al. 2000, Houde et al. 2005, Boas et al. 2006). Contaminant binding to a transport protein not only disrupts the transport of the hormones essential for brain development, but also facilitates transport of these compounds across the blood–brain and placental barriers (de Boer et al. 2000). EDCs may also be able to induce hepatic microsomal enzyme (e.g., uridine diphosphoglucuronosyltransferase, UDPGT) activity (these enzymes mediate extraction or clearance of these endogenous hormones), thereby increasing the excretion of T_4.

Several studies have reported reduced TH and vitamin A concentrations with high PCB concentrations in wildlife species (Brouwer et al. 1989, de Swart et al. 1996, Shaw et al. 1999, Jenssen et al. 2003, Fernie et al. 2005, Sørmo et al. 2005, Tabuchi et al. 2006, Letcher et al. 2010, Schwacke et al. 2012). For example, PCBs were negatively correlated with total T_4 and were positively correlated with TR-α (a thyroid hormone receptor) in free-ranging harbor seal pups from inland waters of Washington and British Columbia (Tabuchi et al. 2006). Similar to the free-ranging harbor seal pups from inland waters, preliminary results also revealed an association between reduced thyroid hormone levels in harbor seal pups from California with increasing levels of PCBs, and lower levels of PCBs were associated with a stronger immune response (Shaw et al. 1999). These studies suggest that harbor seals may be highly sensitive to contaminant-induced endocrine disruption. Correlations between contaminants, TH levels, and TR-α expression can also indicate an increased risk in thyroid hormone-dependent health effects, such as developmental abnormalities. For example, developmental disturbance, measured by fluctuating skull asymmetry in Baltic gray seals (*Halichoerus grypus*), was associated with a heavy pollution period (Zakharov and Yablokov 1990). Gray seals born in the 1960s experienced peak pollution levels in the environment and had higher levels of skull asymmetry compared to seals born prior to the peak in pollution (Zakharov and Yablokov 1990). Although the health status of ringed seals (*Phoca hispida*) from the polluted Baltic Sea has improved, they continue to experience thyroid disruption, changes in vitamin A, and changes in hepatic mRNA expressions (Routti et al. 2010). PCB-induced endocrine disruption has also been observed in cetaceans. For example, bottlenose dolphins off the Georgia coast have severely decreased TH levels that are significantly correlated with extreme concentrations of PCBs, and a high prevalence of anemia (26%) was observed (Schwacke et al. 2012).

Several studies suggest that PBDEs are also potential endocrine disruptors (de Boer et al. 2000, Legler and Brouwer 2003, Darnerud 2008, Legler 2008). Similar to PCBs, PBDEs can interfere with the transport and metabolism of THs and disrupt the TH homeostasis. Because some PBDEs or their metabolites are structurally similar to T_4, they can significantly reduce T_4 concentrations in laboratory species (Zhou et al. 2001, Richardson et al. 2008). For example, a commercial PBDE mixture that contains tetra- and penta-forms (DE-71) caused serum T_4 levels to be reduced in rat fetuses and offspring, and these levels did not recover until approximately 1 month later (Zhou et al. 2002). American kestrel (*Falco sparverius*) eggs and nestlings that were exposed to penta-BDE congeners also had lower plasma T_4 and lower plasma retinol (Fernie et al. 2005; see review in Letcher et al. 2010). It may also be possible that a contaminant-induced reduction of T_4 levels has the potential to alter hearing and communication in mammals (e.g., Crofton 2004).

Sex Hormones

Androgens, estrogens, and progestins are steroid hormones that are primarily synthesized in the adrenals, gonads, and placenta (see reviews in Gregory and Cyr 2003 and Atkinson et al. 2009). More specifically, testosterone is produced in the testes, estrogen and progesterone are produced in the ovaries, and all three can be produced in the adrenals. The hypothalamus produces and secretes gonadotropin releasing hormone (GnRH), which acts on the pituitary gland.

The pituitary stimulates the synthesis and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). In females, FSH regulates the ovarian follicle and the production of estradiol, and LH triggers ovulation and ovarian production of estradiol. In males, FSH promotes spermatogenesis and LH stimulates secretion of testosterone.

Sex steroids in marine mammals are consistently low in concentration prior to sexual maturity, followed by a fluctuation in concentration at the onset of sexual maturation (Atkinson et al. 2009). In some male marine mammals, testosterone concentrations vary depending on the female breeding activity, but typically have peak concentrations prior to the breeding season (Atkinson et al. 2009 and references within). In captive male killer whales, mean serum testosterone concentrations at puberty and at sexual maturity were 0.7 ± 0.7 ng/mL and 6.0 ± 3.3 ng/mL, respectively (Robeck and Monfort 2006). However, unlike seasonally reproductive species that experience clear seasonal peaks in testosterone concentrations, captive male killer whales had "seasonal rhythms" in testosterone, but spermatogenesis was maintained throughout the year (Robeck and Monfort 2006). In male Southern Resident killer whales, fecal testosterone increased asymptotically with the onset of puberty, estimated at 11 to 12 years of age (Ayres 2011).

Progesterone is the primary hormone responsible for sustaining pregnancy in mammals. In fact, pregnancy detection has been successfully attempted by detecting progesterone and other reproductive steroids in several media, including serum or plasma, blubber, feces, saliva, milk, and ocular secretions (Rolland et al. 2005, Atkinson et al. 2009, Kellar et al. 2013, Trego et al. 2013, Wasser et al. in prep.). For example, serum progesterone levels were above 3 ng/mL in pregnant belugas and between 4.3 and 4.5 ng/mL in pregnant and ovulating minke whales (*Balaenoptera acutorostrata*; see Boyd et al. 1999 and references within).

Effects on Reproduction from Organic Contaminant Exposure

Some contaminants can have endocrine-disrupting effects that mimic or alter reproductive processes. Consequently, sex-related hormone alteration and adverse reproductive effects have been associated with contaminant exposure in several studies. For example, high PCB loads have been associated with reproductive failure in several pinniped species. In one study, premature births were found to occur in California sea lions (*Zalophus californianus*) with higher levels of PCBs in the blubber and liver (Gilmartin et al. 1976). PCBs were also suggested as the main cause of the higher frequency of reproductive failures in harbor seals fed fish from the more polluted waters of the western part of the Wadden Sea than seals fed fish from the less polluted waters of the northeast Atlantic (Reijnders 1986). One possible explanation for the reproductive failure observed in the harbor seals is that 17β-estradiol levels were lower around the time of implantation, which may have impaired endometrial receptivity and prevented successful implantation (Reijnders 1986, Reijnders 2003). It was later suggested that the lower levels of estradiol were possibly from enzyme-induced metabolism by PCBs; however, further studies are needed to test this possibility (Reijnders 2003).

Contaminant-induced reproductive impairment (e.g., Subramanian et al. 1987) or failure (e.g., Schwacke et al. 2002) has also been suggested in cetaceans. Reproductive success in bottlenose dolphins, measured by the calf's survival, may be significantly reduced because of chronic exposure to PCBs (Schwacke et al. 2002), corroborating other studies that have measured maternal contaminant burdens and calf mortality rates (Reddy et al. 2001, Wells et al. 2005). However, other confounding factors, such as the mother's age and maternal experience, may have influenced the reproductive success in some of these studies. Population growth rates may also be adversely affected by PCB contamination. For example, an individual-based model was used to estimate the effects of PCB accumulation rates on potential population growth rates in a bottlenose dolphin population (Hall et al. 2006b). Using a dose–response relationship based on maternal PCB burdens and first-year calf survival, the model results suggest that the current PCB accumulation rates may be depressing the population growth rate (Hall et al. 2006b).

Sex determination at birth (or the sex ratio of a population) may have the potential to be affected by EDCs. Because EDCs can mimic reproductive hormones that are thought to influence sex determination processes, it has been suggested that populations with high burdens of these EDCs and poor body condition may exhibit an altered sex ratio toward either females or males. For example, the temperature of egg incubation determines the sex of some reptile hatchlings. However, turtle eggs exposed to PCB concentrations comparable to levels observed in human breast milk showed a significant reversal compared to what would be expected for the temperature in which they were reared (Bergeron et al. 1994). Similar results are shown for birds. For example, female glaucous gulls (*Larus hyperboreus*) with high organochlorine levels and in poor body condition had an unexpected skew toward male offspring (Erikstad et al. 2011). Skewed sex ratios are not isolated to wildlife, but are found in humans as well. For example, maternal exposure to PCB-contaminated fish from the Great Lakes appeared to skew the sex ratio toward females (Weisskopf et al. 2003). A statistically significant decline in male births was also observed in the Aamjiwnaang First Nation community in Ontario, Canada (Mackenzie et al. 2005). Though there is not a significant difference between time periods, in the 1970s, births in the Southern Resident killer whale population were biased toward females, whereas since 2000, the current population has been biased towards males (Ward et al. 2013). Currently, there is little information on maternal or paternal influences on the sex ratio in killer whales. However, recent studies have provided in vitro sperm characterization and characterized the sexual maturation of captive male killer whales (Robeck and Monfort 2006, Robeck et al. 2011), which may be useful in detecting the effects of EDCs on gamete quality. Although there are several biological and environmental factors that can affect or influence a population's sex ratio, and little information is available for marine mammals, the potential for endocrine disruptors to influence the sex ratio warrants further assessment.

Influence of Organic Contaminants on the Nervous System

In mammals, the nervous system is divided into the central nervous system, formed by the brain and spinal cord, and the peripheral nervous system, which includes all the nervous tissues in the body (Pabst et al. 1999). The maturation and development of the central nervous system, also

referred to as neurogenesis, has two main stages. The first stage consists of early embryonic brain development, and the second stage is referred to as the brain growth spurt (Fischer 2008, citing Davison and Dobbing 1968).

The height of the brain growth spurt appears to be the most critical or sensitive period in mammals for developmental neurotoxicity due to contaminant exposure (e.g., Darnerud 2003, Viberg et al. 2003, Viberg et al. 2006, Darnerud 2008). For example, neonatal mice exposed to BDE-99 during a critical period of brain development (day 3 or 10) experienced impaired spontaneous behavior; however, mice exposed on day 19 did not experience the neurotoxic effect (Eriksson et al. 2002). In a separate study, neurobehavior in rats was examined subsequent to in-utero exposure to BDE-99 (Kuriyama et al. 2005). Exposure during critical developmental phases caused hyperactivity in offspring. These studies indicate that adverse health effects from exposure to some PBDE congeners are not only dose-dependent and species-specific, but that the timing of exposure during gestation is a significant factor.

Developmental neurotoxicity is considered one of the greatest concerns for potential adverse health effects from exposure to PBDEs (Costa and Giordano 2007). More importantly stressed is the increased probability of the hydroxylated (OH-) PBDEs over their parent congeners causing developmental neurotoxicity (Dingemans et al. 2011). PBDE exposure can have continuing behavioral alterations (e.g., changes in spontaneous behavior such as locomotion, rearing, and total activity, hyperactivity, and decreased habituation) and cognitive impairment or reduced learning and memory in laboratory species (Eriksson et al. 2001, Viberg et al. 2003, Viberg et al. 2006, Costa and Giordano 2007, Johansson et al. 2008). Very few studies have investigated the neurobehavioral effects of PBDEs in humans; however, in one study, serum levels of PBDEs in adolescence were associated with reduced motor function (Kiciński et al. 2012). Several studies have also revealed effects of PBDEs on brain function and structure, cell viability, cell differentiation and migration, and neuronal signaling (Dingemans et al. 2011). The exposure effects on cell signaling and function have been reported at concentrations similar to, or higher than, PCB exposure effects (Costa and Giordano 2007).

In many species, certain PCB congeners can cause neurological impairment (Porterfield 2000). In both laboratory animal and human studies, exposure to PCBs reduces motor activity, learning and memory, responsiveness, neuromuscular development, and sensory function (see Bowers et al. 2004 for citations). One potential mechanism of neurotoxicity is the disruption of the thyroid system (Porterfield 2000). Because the THs control the development of the central nervous system, abnormal TH levels during specific developmental periods could lead to significant neurological impairment. Hypothyroidism, a reduced production of THs, or hyperthyroidism, an increased production of THs, during development can have profound impacts. During brain development, the effects of PCBs on TH-dependent gene expression were found to be congener-specific (Roelens et al. 2005). Rats dosed orally with a PCB mixture from gestation day 1 to postnatal day 23 had increased mortality and reduced growth, thyroid function, and neurobehavioral development (Bowers et al. 2004).

Influence of Organic Contaminants on Immune Function and Disease Susceptibility

Immune System and Functional Testing Overview

The immune system of killer whales has been studied under the assumption that many of its cellular components are functionally similar to better-described mammalian systems such as mouse, harbor seal, or human. To provide a background, an extremely streamlined overview of the roles and interactions of mammalian immune cells is shown in Figure 5, one that covers only those aspects mentioned subsequently.

Immunity can be coarsely separated into non-specific (or innate) and specific (or acquired) immunity, operationally defined by whether the response is directed against a range of foreign molecules (or antigens) or a specific foreign antigen, respectively. Phagocytic cells, such as macrophages and neutrophils, are often early responders to the presence of foreign antigens. Phagocytes ingest and destroy pathogens using reactive oxygen species and respiratory burst chemistry, present antigens to B cells and T cells, and release molecules that stimulate the proliferation of other immune cells and signal for the recruitment of cells for further immune response (Figure 5). A second important cell type involved in non-specific immunity is the natural killer cells that contact and destroy infected cells by releasing cytotoxic molecules.

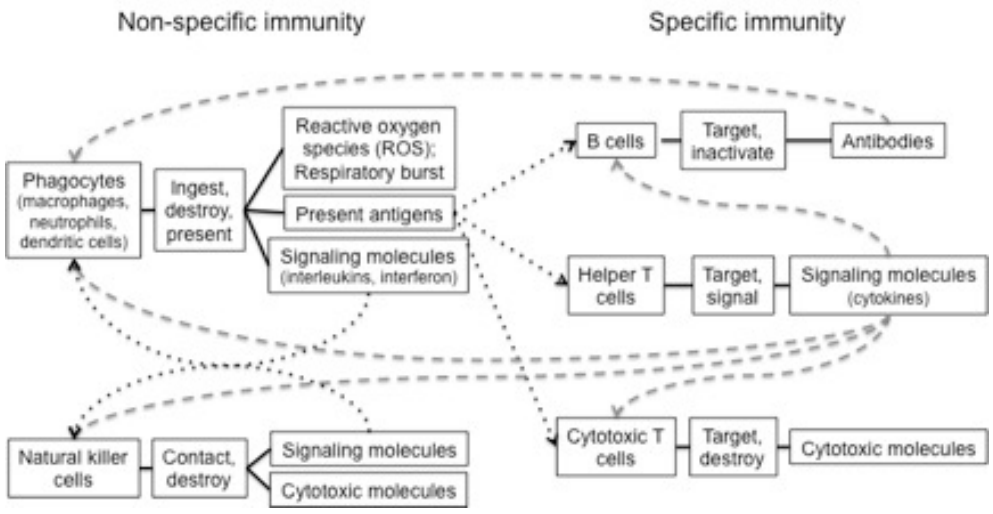

Figure 5. Highly simplified diagram of the types, functions, and products of immune cells involved in nonspecific and specific immunity. Products that influence other immune cells are connected by arrows. Diagram is not comprehensive for immune function observed or studied in marine mammals.

B cells are well-known components of specific immunity because of their role in conferring protection against specific pathogens through antibody production. Antibodies attach to foreign antigens, making them more susceptible to attack by non-cellular parts of the immune system (e.g., the complement system) or by non-specific immune cells such as phagocytes. Cytotoxic T cells operate similarly to natural killer cells, except that they target specific antigens or pathogens. Helper T cells are often considered the lynchpin of the specific immune response, because they not only identify specific foreign antigens but also release signaling molecules that direct further targeted immune response.

Measuring the magnitude and speed of activity of these cellular components has been useful in assessing the competence of the immune system. The majority of these measurements are conducted on tissue that is removed from the organism and either placed into culture conditions (in vitro) or tested without using culture conditions (ex vivo). The function in the animal is inferred from these types of tests.

Immune function in whales and dolphins appears to have strong parallels to that observed in terrestrial mammals, particularly laboratory models (e.g., rodents) and humans. Components of both innate and acquired immunity have been identified, and assays to phenotype or test function have been developed for some of these components. Phagocytosis, respiratory burst (generation of reactive oxygen species), natural killer cell activity, and non-specific lymphocyte transformation are signature features of an innate immune response, and assays were adapted or modified for cetaceans as early as 1975 (Hrgovcic et al. 1975). Immune phagocytosis and respiratory burst are typically mediated by neutrophils and macrophages. Bottlenose dolphin neutrophils exhibit NADPH oxidase activity (Inoue et al. 2001), and sequence alignments of NADPH oxidase in neutrophils have significant homologies to ruminant molecules (Egawa et al. 2001). Functional markers of T cells, B cells, histiocytes, and macrophages have been identified through cross-reacting antibodies from humans, mice, cattle, and sheep, indicating that homologous immune subsets are present in whales and dolphins (Kumar and Cowan 1994, Shirai et al. 1998a, Shirai et al. 1998b, Beineke et al. 2001, Jaber et al. 2003a, Jaber et al. 2003b, Kawashima et al. 2004a, Kawashima et al. 2004b, Kawashima et al. 2004c, Komohara et al. 2006). Use of these and cetacean-specific antibodies directed to recognize lymphocyte and leukocyte subsets support the functional role of the markers by comparing healthy and diseased animals. For example, harbor porpoises suffering from suppurative lesions exhibited selective depletion of $MHCII^-$ cortical thymocytes, thymic medullary $CD45R^+$ B cells, and splenic periarteriolar $CD3^+$ lymphocytes (Beineke et al. 2007).

Cytokines are the primary soluble and membrane-associated signaling molecules of the immune system, and a spectrum of important and well-characterized cytokines have been isolated and sequenced (see Table 1 in Beineke et al. 2010). Sequence homologies to human and terrestrial mammalian cytokines typically occur within regions of biological activity, permitting an inference of function. However, better support for homologous activity is found in studies that demonstrate cross-species activity. For example, recombinant cetacean TNFα and IL-1β have been used to stimulate terrestrial mammalian cell lines (Inoue et al. 2001, Shoji et al. 2001). Conversely, cetacean NK cell activity can be potentiated with recombinant human IL-2 (de Guise et al. 1997).

Whole genome sequencing of the killer whale genome[1] (by the Baylor College of Medicine Human Genome Sequencing Center) and comparative analysis of killer whale genomes (Moura et al. 2014) are uncovering homologous immune genes that have been characterized in model organisms, such as mice. Genes encoding proteins ranging from non-specific (e.g., complement, C-reactive protein) to specific immune functions (e.g., interleukin-12 and granulocyte–macrophage colony stimulating factor 2) have been identified. Furthermore, gene orthologs for surface molecules involved in mediating cell adhesion associated with inflammation, such as e-, l-, and p-selectins,[2] as well as other cell surface molecules that modulate host response (e.g., integrins), have been identified. The homology with well-characterized mammalian genes suggests that killer whale immune function is likely to follow known mechanisms of immunity.

Effects on Immune Function of Organic Contaminant Exposure

Several POPs have well-documented effects on the immune system in experimental animals ranging from mice to primates (e.g., Thomas and Hinsdill 1978, Thomas and Hinsdill 1980, Safe et al. 1989, Dahlman et al. 1994). Dioxin-like POPs are particularly effective at immunotoxicity across a range of species, e.g., affecting the formation of reactive oxygen species (ROS) in neutrophils (Fonnum et al. 2006) or reducing mitogen-stimulated lymphocyte proliferation (de Swart et al. 1996). Effects can also be exerted indirectly by modulating immune function, such as reducing transfer of maternal antibodies to offspring (Lyche et al. 2006).

In vitro and ex vivo studies are often used to provide insights into potential effects on cellular functions of the immune system. In vitro experiments are typically conducted by exposure of immune cells to contaminants in a culture dish, and ex vivo experiments utilize immune cells isolated from animals with varying levels of tissue contaminants. Exposure of harbor seal peripheral blood leucocytes (PBLs) to PCBs can induce a reduction in phagocytic activity and respiratory burst, suggesting possible suppression of a non-specific immune response (Hammond et al. 2005). Similarly, exposure of PBLs from harbor seals to three different PBDE congeners resulted in reduced phagocytic activity and efficiency and a decrease in intracellular thiol, but a slight increase in ROS, a measure of respiratory burst (Frouin et al. 2010). Real-world exposures are usually mixtures of congeners, and even within the reduced complexity environment of in vitro exposures, non-specific and specific cellular responses vary with mixture (Levin et al. 2007), emphasizing that immunotoxicity can have both elevating as well as suppressive effects on immune function. A seminal feeding study demonstrated significant immunomodulation of immune responses in harbor seals fed herring that had either high or low concentrations of PCBs and DDTs. Natural killer cell cytotoxicity and mitogen-stimulated lymphoproliferation were reduced for seals fed highly contaminated herring for 93 weeks, implying a lowered ability to contain viral infections (de Swart et al. 1994). Furthermore, the specific proliferative responses to immunizing antigens and delayed type hypersensitivity were also reduced, particularly during the latter half of the exposure period, indicating that more complex interactions among immune cell

[1] http://www.ncbi.nlm.nih.gov/bioproject/167475
[2] http://www.ncbi.nlm.nih.gov/gene/?term=orcinus+orca+AND+selectin

types were also impaired (de Swart et al. 1995). Interestingly, B cell responses such as LPS-induced proliferation and antigen-specific antibody production were not affected, and total white blood cell counts and the percentage of granulocytes in the peripheral circulation were increased (de Swart et al. 1994, de Swart et al. 1995).

Microscopy and hematology are time-honored techniques for assessing immune potential, and histological examinations can reveal integrated impacts of an immunocompromised state. In harbor porpoise that were either by-caught or stranded and had high levels of PCBs and PBDEs, severe lymphoid depletion in the thymus and spleen were observed (Beineke et al. 2005). The thymus, a primary lymphoid organ, is the source of T cells, while the spleen, a secondary lymphoid organ, is a site where activated T and B cells are localized to advance an immune response. Lymphoid depletion in the thymus was associated with elevated PCB levels, whereas depletion in the spleen was associated with increased PBDEs. Lymphoid depletion may also be associated with persistent stress and malnutrition, as well as bacterial and viral infections. A hematology survey of harbor seal in San Francisco Bay identified a positive correlation between total peripheral leucocytes and concentrations of PCBs, DDE, and PBDEs, but a negative correlation between total red cell counts and concentrations of PBDEs, indicating a potential for anemia with exposure (Neale et al. 2005).

Disease Susceptibility

Killer whales have been diagnosed with a spectrum of infectious and non-infectious diseases (Appendix A and Appendix B), and these animals are exposed to a large array of pathogens in the aquatic environment. Some pathogens and pollutagens (anthropogenic pathogens, chemicals, and molecules) are derived from terrestrial sources, some are inherent to marine waters, some are commensals or compose part of the normal microbial flora of the animals, and some are found in humans and sympatric marine mammals (Appendix C). The preponderance of data indicates that POPs exert an immunomodulatory effect on cetaceans and pinnipeds proportional to their concentration in tissues. The effects of PBDEs and DDTs are considered less severe than those of PCBs.

A common approach to assessing the association between contaminants and disease susceptibility has been to compare tissue contamination levels to morbidity, to the degree of inflammation (host response), or to infection (e.g., Isobe et al. 2011). Furthermore, chronic disease or infection can lead to emaciation, which may mobilize contaminants, increase exposure, and exacerbate a disease condition. Although nematode infections in stranded harbor porpoises between 1989 and 2002 in the United Kingdom were positively correlated with blubber PCB levels, the authors noted that controlling factors, such as age, gender, and cause of death, were not included in the analysis (Bull et al. 2006). A wide geographic comparison of POP concentrations by cause of death (infectious vs. non-infectious) found significantly higher levels of PCBs and PBDEs in harbor porpoise that died from infections (Pierce et al. 2008). This association was not observed for the bottlenose dolphin, and the authors suggested that the low number of dolphins that died from infections (12 of 716) severely reduced the power of the comparison. An ad hoc analysis of the relationship between PCB concentrations in the blubber of harbor porpoises and cause of death (physical trauma, infectious disease) found higher levels of contaminants

among animals that died from infectious disease, even when controlled for gender (Jepson et al. 2005). In contrast, a study of California sea otter (*Enhydra lutris*) that classified cause of death into four categories (infectious disease, emaciation, trauma, and other) failed to identify a convincing association between infectious disease and concentrations of industrial POPs (PCBs and PBDEs) in the liver (Kannan et al. 2007). Unfortunately, it is not possible with these studies to determine whether the observations are species-specific or caused by particular combinations of POP congeners.

The occurrence of neoplasms, or tumors, associated with exposure to POPs may represent a combination of mutagenic effects with immunotoxicity, immunosuppression, and sustained exposure, because of the resistance of POPs to degradation or excretion. The longer time required for neoplastic development makes it difficult to define a mechanistic relationship. However, tumor induction in laboratory mice exposed to DDT over their lifetime, as well as in mice exposed to DDT for 15–30 weeks, demonstrates the neoplastic potential of POPs (Turusov et al. 2002). California sea lion with herpesvirus-induced metastatic carcinoma had higher levels of blubber PCBs than animals without carcinoma, even when controlling for blubber thickness (Ylitalo et al. 2005). Similarly, St. Lawrence belugas had much higher PCB concentrations than Arctic belugas and had a higher occurrence of tumors and lesions, and some evidence of immunosuppression (Béland et al. 1993, Martineau et al. 1994). Even among humans, a significant risk of breast cancer associated with serum POP levels can be detected among certain populations through case–control study methods (Bonefeld-Jørgensen et al. 2011), suggesting that POPs may contribute to carcinogenesis in multiple ways.

The Development of Biomarkers for Toxicity

Biomarkers can potentially signal whether a population is at risk from contaminant exposure. They are generally classified as biomarkers of exposure or biomarkers of effects. Biomarkers of exposure do not give an indication of a toxicological effect, but can provide early evidence of exposure in an individual (e.g., BPMO induction in marine mammal skin biopsies; Fossi et al. 1999). More biomarkers need to be developed and validated for marine mammals, but there are several potential biomarkers that have been investigated and proposed, including induction of the cytochrome P4501A enzyme system, plasma TH and vitamin A levels, immune responses, morphologic lesions, and assessment of DNA damage. The interpretation of these biomarkers' responses to contaminant exposure is challenging, however, because of the numerous factors that can influence the endocrine, nervous, and immune systems. Furthermore, there is still a large gap between observing a biomarker response and understanding how this response affects the animal or population (O'Hara and Becker 2003). Here we provide a few examples of proposed biomarkers of exposure and biomarkers of effects for marine mammals.

The cytochrome P4501A enzyme system is the primary biochemical pathway for metabolizing contaminants and is an established biomarker for environmental pollution levels. The induction of this system has been used for dioxin-like congeners (Ross and Birnbaum 2003, Angell et al. 2004). The dioxin-like congeners are toxic, and can interact with the aryl hydrocarbon receptor (AhR) and stimulate the synthesis of cytochrome P450 (CYP450). In general, a dioxin-like molecule enters the cell and can bind to the AhR (Figure 6). The AhR-contaminant complex migrates into the nucleus and alters gene expression. This then leads to the transcription of mRNAs that induce forms of CYP450. Inducing these enzymes can lead to the metabolism of these dioxin-like congeners and the formation of more toxic metabolites (Darnerud 2008). Thus, the AhR is a key regulator of the cellular response to contaminant exposure. After these compounds are chemically transformed by hepatic biotransformation processes, the more hydrophilic metabolites circulate in the blood of marine mammals and are more readily excreted from the body (Houde et al. 2005).

The induction of cytochrome P4501A can occur within hours of exposure to contaminants, and will decrease when metabolism or excretion of the contaminant occurs (Angell et al. 2004). Therefore, the levels of P4501A are an early contaminant-induced biological response (Angell et al. 2004). However, the identification of other potential influential factors of P4501A levels, such as the species, sex, age, diet, migration, reproductive status, and sampling location along the body, is essential to properly interpreting P4501A expression (Angell et al. 2004). In a previous study, van den Brink et al. (2000) proposed that the pattern of PCB congeners in the blood was a potentially nondestructive biomarker, linking the induction of cytochrome P450 activity to PCB exposure.

Other biomarkers for contaminant-associated toxic effects include measurements of circulating vitamin A in the peripheral blood, the expression of the vitamin A receptor RARα, and levels of circulating THs. Vitamin A is essential for growth, development, immunity, and reproduction. Ingested vitamin A is primarily stored in the liver and then transported to other tissues by the retinol binding protein (RBP) along with the transport protein TTR. In general, these two

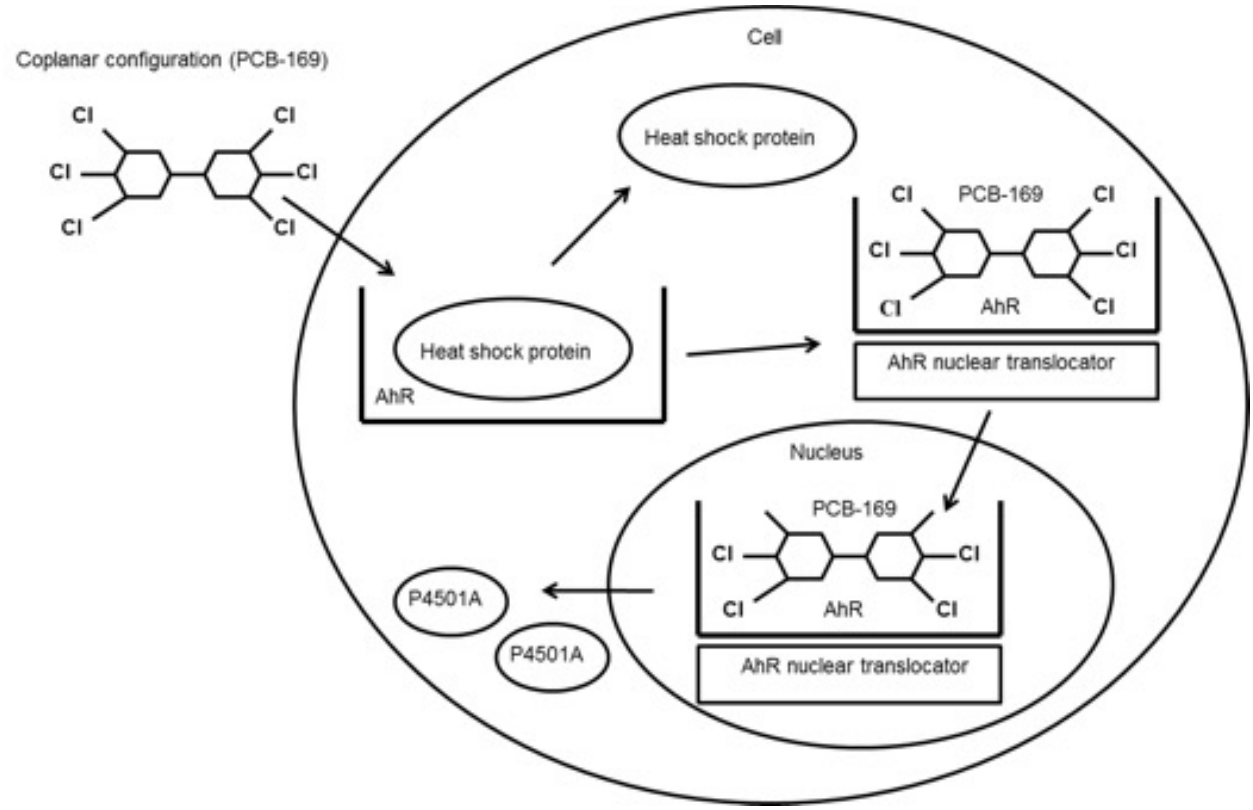

Figure 6. Diagram showing the interaction between an aryl hydrocarbon receptor (AhR) and a dioxin-like compound. The dioxin-like compound (PCB-169) enters the cell and binds with AhR. The inducer–receptor complex translocates to the nucleus, where the transcription of specific mRNA occurs. The result is the induction of enzymes (cytochrome P4501A).

proteins maintain a constant concentration of circulating vitamin A. Although there is strict regulation of vitamin A in the body, some PCB and PBDE congeners can interfere with its transport, metabolism, and storage, ultimately reducing circulating vitamin A levels (Rolland 2000, Hallgren et al. 2001, Mos et al. 2007). Several studies have also shown that PCBs and PBDEs can reduce T_4 levels, although PCBs may have a stronger effect on reducing T_4 levels than PBDEs (e.g., see Hallgren and Darnerud 2002). The interpretation of the reduction in vitamin A and thyroid hormone levels and increased levels of POPs must include a consideration of the confounding factors, such as season, sex, age, and life history. For example, Simms et al. (2000) found that age and lactation status may interfere with interpreting the effects of PCBs on circulatory vitamin A levels in harbor seals. In contrast to other studies, PCBs and vitamin A were positively associated in non-nursing harbor seal pups, and this association was attributed to vitamin A stores from the liver becoming mobilized following contaminant exposure (Simms et al. 2000).

It was previously thought that corpora albicans, or ovarian scars, in some cetaceans accumulate with age and can provide individual lifetime reproductive histories (Perrin and Donovan 1984). Thus, with an index of reproductive history, one may assess the effects of contaminants on reproductive output. This assumption is not without controversy. Dabin et al. (2008) examined age, reproductive history, and ovarian scars in a relatively large sample (n = 187) of short-beaked common dolphins (*Delphinus delphis*). They found that the numbers of scars present in ovaries

did not increase with age after sexual maturity. Thus, counting ovarian scars to reconstruct reproductive histories may have limited potential in this species, and the authors suggest that the validity of this assumption for other cetaceans should be reassessed as well (Dabin et al. 2008). More recently, Murphy et al. (2010) investigated this question (whether ovarian scars accumulate and are persistent), and also investigated the potential to use these scars as an index of reproductive activity in female common dolphins and the harbor porpoise from the eastern North Atlantic. They found that the number of scars in porpoise ovaries was significantly related to age up to around five years (soon after sexual maturity); in common dolphins, the number of scars increased until around age 12 (sexual maturity). Furthermore, Murphy et al. (2010) investigated POP effects on the reproductive activity in both species using ovarian scars as an index of reproductive activity. Higher PCB concentrations in harbor porpoises tended to be associated with low numbers of ovarian scars (Murphy et al. 2010). Common dolphins had PCB levels above a threshold level (17 μg/g lw [lipid weight]) for adverse health effects in marine mammals (Kannan et al. 2000); however, ovulation, conception, or implantation were not inhibited (Murphy et al. 2010). The highest PCB burdens in the dolphins were found in mature females (non-lactating and non-pregnant), who had the highest number of ovarian scars. These conflicting results warrant more research on the potential to use ovarian scars in certain species as an index of reproductive history and an assessment of the effects of contaminants on reproductive output.

Direct measurement of the immune responses can offer informative biomarkers for POP immunotoxicity (de Guise et al. 2003). Cellular measures of immune function, such as phagocytic capability and induction of lymphocyte proliferation in vitro, readily detect certain POP-associated impairments of immune function (Levin et al. 2005, Schwacke et al. 2012). The advent of molecular techniques to measure gene expression at the level of the RNA transcript allows assessment of immune signaling molecules, such as IL2, IL10, and interferonγ (Beineke et al. 2010). However, to make an appropriate interpretation, these results should be interpreted with an understanding of contaminant loads and patterns associated with gender, age, and/or physiological status, such as pregnancy or lactation (Frouin et al. 2010). Because these molecules are components of a complex suite of changes, no single molecule or cellular response can serve as an immune biomarker of POP exposure. Furthermore, factors such as chronic stress or nutritional deficiencies can also induce changes in immune function.

Several recent studies have described gene expression and DNA damage as useful biomarkers of toxicity. Buckman et al. (2011) observed a positive correlation between ΣPCB concentrations in killer whale blubber and the expression of five gene targets: AhR, thyroid receptor α, estrogen receptor α, interleukin 10, and metallothionein 1. Their results strongly suggest that PCBs influence mRNA abundance or expression for these five genes (Buckman et al. 2011), and that these gene targets are likely potential biomarkers of toxicity. El-Zein et al. (2006) compared levels of DNA damage and apoptosis in California sea lion and human lymphocytes in response to two different toxicants, and found the comet assay to be a useful biomarker of contaminant-related effects. Fossi et al. (2010) analyzed a combination of several molecular biomarkers (CYP1A1 and CYP2B) and levels of gene expression (qRT-PCR of HSP70, ERα, AHR, and E2F-1) with contaminants (PCBs, PBDEs, DDTs, and PAHs) in two fin whale populations. Their "multi-trial diagnostic tool" revealed that fin whales from the Mediterranean Sea (Pelagos Sanctuary) have a higher toxicological stress than fin whales from the Gulf of California (Sea of Cortez, Mexico), providing further support for the usefulness of these biomarkers.

Interactive Effects

Killer whales are exposed to a mixture of contaminants, and the interactions of these contaminants have the potential to be additive, synergistic, or antagonistic. The effect of an additive interaction of two or more chemicals equals the sum of the effects of the isolated chemicals. The effect of a synergistic interaction is greater than the sum of the effects of the individual chemicals, whereas the effect of an antagonistic interaction is less than the sum of the effects of the chemicals separately. Only recently have studies examined the interactive effects of mixtures containing POPs (some examples are provided in Table 5), and we provide a brief review of some of these studies below.

PCBs and PBDEs

Some PCB and PBDE congeners can interact and enhance developmental neurobehavioral defects in laboratory species. For example, Eriksson et al. (2006) found that combined low doses of the congeners PCB-52 and BDE-99 interacted in neonatal mice and enhanced developmental neurobehavioral defects when the exposure occurred during the critical brain growth development. Although it is not certain that the effects were synergistic, the authors suggest that the effects of the interaction were more than just additive. The developmental neurotoxic effects of these two congeners included deviations in spontaneous behavior (i.e., locomotion, rearing, and activity) and habituation capability (defined as a decrease in spontaneous behavior over the trial period because of the diminishing novelty of the test chamber), and indicated a progression towards brain dysfunction. In addition, the habituation significantly deteriorated with advancing age in the mice exposed to the mixture, as well as in mice exposed to a higher dose of PCB-52. Developmental neurotoxic effects in the mice that received the combined low dose of PCB-52 and

Table 5. Mixture content and interaction type, with corresponding health effect(s), from case studies.

Mixture	Interaction Type	Health Effect(s)	Reference(s)
PCB-52 + BDE-99	additive and/or synergistic	enhanced neurobehavioral defects	Eriksson et al. (2006)
PCB-153 + BDE-47	additive and/or synergistic	enhanced developmental neurotoxicity and cytogenotoxicity	Gao et al. (2009), He et al. (2009), He et al. (2010)
PCB mixture + BDE-47 + CPs	additive and/or synergistic	TH disruption, induced enzyme activity	Hallgren and Darnerud (2002)
PCB-126 + MeHg	additive	enhanced developmental neurotoxicity	Fischer (2008)
PCB-153 + MeHg	additive and/or synergistic	enhanced developmental neurotoxicity	Fischer et al. (2008a), Fischer (2008)
BDE-99 + MeHg	additive and/or synergistic	enhanced developmental neurotoxicity	Fischer et al. (2008b), Fischer (2008)
PCB + PBDE	additive	reduced T4 levels	Miller et al. (2012)
BDE-47 or BDE-99 + PCB-126 or PCB-153	additive, synergistic, and/or antagonistic	cytotoxicity	Pellacani et al. (2014)

BDE-99 were significantly more pronounced than in mice that received a five-fold higher dose of PCB-52 alone. Moreover, no significant change in spontaneous behavior was observed in mice that were exposed only to the same low dose of PCB-52 or BDE-99, indicating that toxicity occurs at a lower dose when these two congeners are combined.

PCB and PBDE congeners can also interact and induce protein and mRNA expression and reduce learning and memory in laboratory species. For example, He et al. (2009) examined the learning and memory of 2-month-old rats neonatally exposed to a single dose of either a mixture of PCB-153 and BDE-47 or of each congener individually. The results suggest that rats co-exposed to both congeners performed significantly worse on a learning and memory test than rats exposed to BDE-47 alone. BDE-47 induced neurotoxic effects by affecting the learning and memory in a dose-dependent manner. In contrast, both additive and synergistic reactions were observed in inducing protein and mRNA expression in female and male rats. Lastly, several alterations were observed in the neuronal ultrastructure in rats exposed to the high dose of BDE-47 and the mixtures containing both congeners. Therefore, the results suggest that the mechanism of action was via damaging the neurons of the hippocampus (the hippocampus has been associated with memory and learning).

These same congeners (PCB-153 and BDE-47), which can induce protein and mRNA expression and reduce learning and memory in rats, have also been observed to enhance neurotoxicity and cytogenotoxicity in human neuroblastoma cells in vitro (Gao et al. 2009, He et al. 2010). He et al. (2010) found that at both the lower nontoxic concentrations and the higher toxic concentrations, PCB-153 enhanced the cytotoxicity of BDE-47. The enhanced cytogenotoxicity was evident by enhanced DNA and chromosome damage, significant inhibition of cell division, and DNA–protein crosslink (DPC) formation. There was a greater increase of DNA and chromosomal damage in co-exposures than from the single congener alone. Their results indicate that the effect of the mixture is more than simply additive at specific concentrations. PCB-153 and BDE-47 alone did not significantly inhibit cell survival; however, cell viability was significantly inhibited when co-exposure occurred.

Pellacani et al. (2014) investigated the cytotoxic effects of the combined exposure of either BDE-47 or BDE-99 and PCB-126 (a dioxin-like congener) or PCB-153 (a non-dioxin-like congener). The non-dioxin-like congener (PCB-153) interacted synergistically with both PBDE congeners. Interestingly, the lowest dose of PCB-153 and the highest dose of BDE-47 had an antagonistic effect. The interaction between BDE-47 and the dioxin-like congener (PCB-126) was additive; however, synergistic effects were observed at the higher concentration of BDE-47. The interactions between BDE-99 and PCB-126 were complex, with antagonistic, additive, and synergistic interactions all observed. These results suggest that the nature of the interactions is related to the structure of the PCB molecule, and that the types of interactions vary depending on the concentrations.

PCBs, PBDEs, and Chlorinated Paraffins (CPs)

In another study, Hallgren and Darnerud (2002) examined the effects of exposure to BDE-47, a PCB mixture (Aroclor 1254), and a chlorinated paraffin (CP; Witaclor 171P) on thyroid hormones in seven-week-old female rats. Free T_4 was considered to be the most sensitive parameter to indicate irregularity in thyroid hormone status. Aroclor and the highest dose of

BDE-47 significantly reduced free T_4 levels when administered alone, whereas all mixture groups significantly reduced free T_4 levels. In fact, BDE-47 + Witaclor showed a clear synergistic effect on free T_4 levels. The additive effects to T_4 levels were generally seen in all scenarios. The induction of enzyme (EROD, MROD, and PROD) activity was also determined. These enzymes transform the parent PCBs and PBDEs into their metabolites, which can affect the levels of circulating T_4. EROD activity is a marker for CYP 1A1 activity (mediated via AhR). Therefore, BDE-47 (a non-planar molecule) was not expected to induce such activity. However, BDE-47 + Witaclor had a synergistic effect on EROD activity. The induction of EROD can increase the contaminant's effect, further enhancing the production of metabolites that bind to TTR, thereby decreasing plasma T_4 levels. Hallgren and Darnerud (2002) found that decreases in T_4 levels are primarily due to disruption in the serum transport caused by the binding of the self-induced PCB and PBDE metabolites to TTR. The most pronounced effect of reduced TTR binding was from the BDE-47 + Aroclor group, followed by the BDE-47 + Witaclor group. As a consequence of the synergistic effects observed on T_4 levels and EROD activity, the investigators emphasized the importance of including interactive effects in risk assessment. The thyroid glands were also examined following exposure to the combined mixture of all three contaminants, or the mixture containing BDE-47 and Aroclor, and showed highly active glands (control groups showed low-activity glands).

PCBs, PBDEs, and Methyl Mercury (MeHg)

Fischer (2008) investigated the neurotoxic effects of PCBs, PBDEs, and methyl mercury (MeHg) on neonatal mice co-exposed during the period of rapid growth and development of the brain, or the brain growth spurt. Co-exposure to PCBs (both the ortho-substituted non-dioxin-like PCB-153 and the coplanar dioxin-like PCB-126) and MeHg enhanced developmental neurotoxicity, manifested as defective spontaneous behavior, reduced habituation, learning and memory dysfunction, and alterations in the cholinergic system. Additionally, the neonatal mice co-exposed to PCB-126 and MeHg demonstrated changes in spontaneous behavior that increased over time. Synergistic interactions were observed for PCB-52 + BDE-99, PCB-153 + MeHg, and BDE-99 + MeHg, whereas the co-exposure of the more toxic PCB-126 and MeHg appeared to have an additive effect. Co-exposure to PCB-126 or BDE-99 (same molar dose) and MeHg also reduced nicotinic receptor density in the cerebral cortex and hippocampus, respectively. Most importantly, the observed mixture effects often occurred at low doses, where the individual congeners alone at similar low doses did not cause an effect.

(Anti)Estrogenic Compounds

An estrogenic compound can mimic a hormone, bind to the receptor, and activate a hormone-like response, whereas an antiestrogenic compound acts as an antagonist, potentially binding to a receptor and blocking natural hormones from activating a hormone-like response (O'Shea 1999). There appears to be a wide range of (anti)estrogenic compounds (Table 6). The majority of the PBDE congeners, and two of the three PBDE metabolites tested for their (anti)estrogenic activities, showed estrogenic activity (Meerts et al. 2001). The T_2- and T_3-like metabolites showed the highest estrogenic activity of all the PBDE compounds tested, whereas the T_4-like metabolite

showed no estrogenic effect. In contrast, PCBs have been reported to include both antiestrogenic and estrogenic responses (Buchanan et al. 2000, Bonefeld-Jørgensen et al. 2001, Buchanan et al. 2002, Oenga et al. 2004, Plíšková et al. 2005, Oh et al. 2007).

Exposure to a mixture of contaminants containing contrasting toxicities (e.g., a mixture of estrogenic and antiestrogenic compounds) may mediate the effects of these contaminants. For example, the antiestrogenic compounds PCB-138 and -180 reduced the proliferative effects of the estrogenic compounds 4,4'-DDE and trans-nonachlor, when mixed together (Yordy et al. 2010a). In fact, the combination of the antiestrogenic congener PCB-138 with the estrogenic mixture 4,4'-DDE and trans-nonachlor reduced the estrogenic response (i.e., reduced the proliferative effect), whereas the combination of PCB-180 and the estrogenic mixture resulted in a mixture that was no longer estrogenic.

Table 6. A list of some (anti)estrogenic PCB and PBDE congeners.

PCB-	Estrogenic	Antiestrogenic	Reference(s)[a]	PBDE-	Estrogenic	Antiestrogenic	Reference(s)[a]
28	X		3	28	X		6
44	X		1, 5	30	X		6
49	X		1, 5	32	X		6
52	X		1, 3, 4, 5	47	X		6
66	X	X	1, 3, 5	51	X		6
74	X	X	1, 3, 5	71	X		6
77		X	1, 5	75	X		6
95		X	5	85	X		6
99	X		1, 3	99	X		6
101	X		1, 5	100	X		6
105	X	X	1, 3, 5	119	X		6
110		X	5	153		X	6
118		X	1, 4, 5	166		X	6
128		X	1, 5	190		X	6
138		X	1, 2, 3, 4, 5				
141		X	5				
153		X	1, 2, 3, 4, 5				
156		X	5				
167		X	5				
170		X	1, 3, 5				
174	X		5				
177	X		5				
180		X	1, 2, 3, 4, 5				
187	X	X	1, 3, 5				
194		X	3				
199		X	3				
201	X		5				
203		X	1, 3				

[a] References: 1) Wolff et al. (1997), 2) Bonefeld-Jørgensen et al. (2001), 3) Plíšková et al. (2005), 4) Oh et al. (2007), 5) Yordy et al. (2010a), 6) Meerts et al. (2001).

Summary

In summary, certain mixtures can have interactive effects and enhance toxicity, including enhancing developmental neurobehavioral defects, inducing protein and mRNA expression, reducing learning and memory, and enhancing neurotoxicity and cytogenotoxicity. If co-exposure to a mixture occurs during the sensitive brain growth development, individuals can be more susceptible to toxic effects. Of particular importance is that enhanced toxicity can sometimes occur at lower doses once chemicals are combined, including at concentrations that would be nontoxic for the isolated chemicals. Interestingly, interactive effects in these case studies were additive, synergistic, and/or antagonistic. It appears that the molecular structure of the PCB congener may influence the type of interaction in some cases, and warrants further research. Lastly, mixtures can contain contrasting toxicities that may help mediate the effects, but make it extremely difficult to predict the effects of mixtures on killer whales.

Implications for Southern Resident Killer Whales

Southern Resident killer whales carry relatively high POP concentrations in their blubber (Ross et al. 2000; Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). POP concentrations in Southern Residents are mostly dependent on regional inputs, the distribution of prey items, and the primary foraging area of the whales, which differ between the pods. Here we discuss the three primary POPs (PCBs, PBDEs, and DDTs) that have been analyzed from biopsy samples of Southern Resident killer whales (Figure 7).

The majority of Southern Resident killer whales that have been sampled for biopsy have blubber ΣPCB concentrations that exceed 17,000 ng/g lw (lipid weight), a health effects threshold in harbor seals. However, PCB exposure may have declined over the most recent decades (Ross et al. 2000, Krahn et al. 2007a, Krahn et al. 2009). Ross et al. (2000) biopsied male killer whales between 1993 and 1996 and reported an average ΣPCBs of 146,000 ng/g lw (± 32,700). Krahn et al. (2007a) reported an average ΣPCBs in male killer whales biopsied in 2004–2006 of 66,000 ng/g lw (± 26,000). The apparent reduction in concentration may be due to several factors, including a reduced exposure to PCBs. Krahn et al. (2007a) also suggest it may be due to a difference in methodologies between these two studies (e.g., Ross et al. [2000] summed 136 PCB congeners, whereas Krahn et al. [2007a] summed only 45 congeners). The reduced average blubber ΣPCBs in male killer whales may also be due to the influence of age; the male killer whales biopsied in the more recent study were on average 10 years younger than the whales in the earlier study. Using the same methodology as Krahn et al. (2007a), the Northwest Fisheries Science Center found an average blubber ΣPCBs concentration of 45,000 ± 31,000 ng/g lw in male killer whales sampled between 2004 and 2013.

Although there is an apparent decrease in average PCBs in male Southern Residents, these killer whales carry substantially higher PCB body burdens than the Alaska residents (Krahn et al. 2007b) and Northern Residents (Ross et al. 2000). In fact, some calves have ΣPCB concentrations in blubber that exceed the total PCBs health effects threshold for seals by factors of 2–3.6 (Krahn et al. 2009). Transient killer whales from Alaska (n = 15) and California (n = 4) have even higher blubber PCB concentrations—120,000 ± 49,000 and 630,000 ± 190,000 ng/g lw, respectively—more than double the values derived from Southern Resident killer whales (Krahn et al. 2007b).

PBDEs are also found in relatively high levels in Southern Resident killer whales (Figure 7). Most sampled individuals in the Southern Resident population had higher ΣPBDE concentrations than levels associated with altered thyroid hormone levels in post-weaned and juvenile gray seals (Hall et al. 2003, Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). In fact, one juvenile killer whale had 10 times higher ΣPBDE blubber concentrations than those associated with endocrine disruption in gray seals (Hall et al. 2003, Krahn et al. 2007a). This may not necessarily reflect a unique anomaly, because all of the young juvenile Southern Residents biopsied to date have the highest PBDE concentrations measured in this population (Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). This observation may be due to their small body size and high exposure from nursing, but it also may be related to their reduced capacity to metabolize these pollutants.

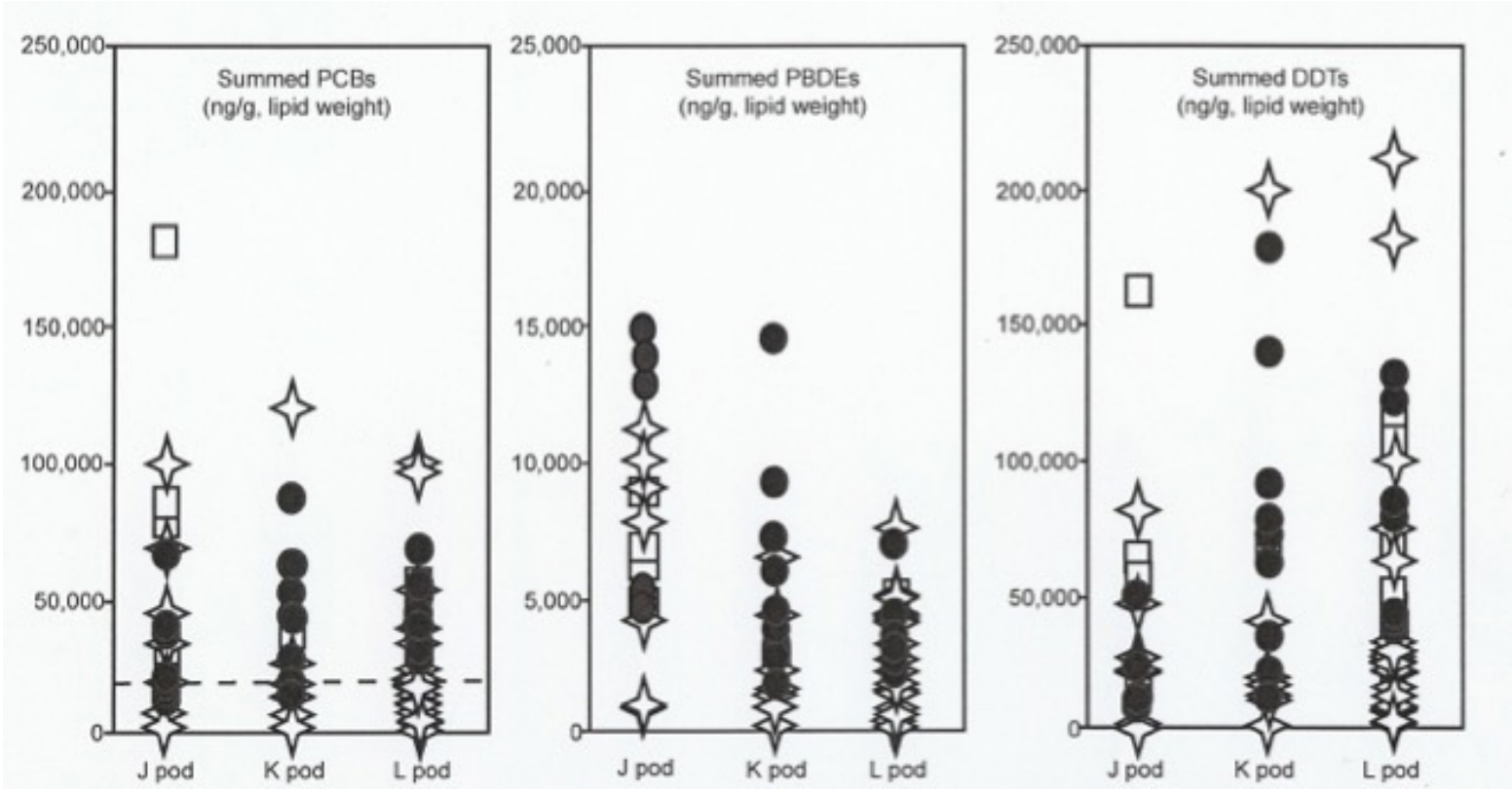

Figure 7. Summed PCBs, PBDEs, and DDTs in the blubber of Southern Resident killer whales. Charts were generated using data from Krahn et al. (2007a), Krahn et al. (2009), and NWFSC unpubl. data. The squares are maturing adult males 13 years and older. The stars are adult females 12 years and older. The black circles are juveniles and sub-adult whales. The dashed line represents the adverse health effects threshold for other marine mammals.

Average ΣPBDE concentrations in the blubber of Southern Residents sampled between 2004 and 2013 was 4,800 ± 3,500 ng/g lw. A higher mean ΣPBDE value (7,600 ± 4,200 ng/g lw) was measured in J pod than the mean levels determined for K (5,000 ± 4,100 ng/g lw) or L (3,500 ± 2,000 ng/g lw) pods, indicating that J pod likely consumes prey that remains closer to urban settings, a primary source of PBDEs (Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). These average levels are higher than those found in other resident populations, as well as levels observed in Alaska transients (Krahn et al. 2007b). According to Rayne et al. (2004), between 1993 and 1996, average ΣPBDE concentrations in the blubber were approximately similar in male Southern Resident killer whales (942 ± 582 ng/g lw) and male transient killer whales from the northeastern Pacific Ocean (1,015 ± 605 ng/g lw). Although transients forage at a higher trophic level than Southern Residents, the proportion of time transients spend foraging on prey that remain closer to urban settings (i.e., Puget Sound) is relatively less than the time J pod spends foraging in urban waters. However, one West Coast transient killer whale sampled off California had substantially higher ΣPBDE blubber levels (12,600 ng/g lw), similar to levels measured in juvenile Southern Residents (Krahn et al. 2007b, Krahn et al. 2009).

PBDE concentrations in the Southern Residents have likely increased exponentially, with a doubling time between 3 and 4 years (Mongillo et al. 2012). However, based on other species that have experienced declining PBDE levels (Elliott et al. 2005, Law et al. 2010, West et al. 2011, Ross et al. 2013), and because of the decrease in production and use of certain PBDE technical mixtures, this increase of PBDEs in Southern Residents is expected to slow, and we anticipate declining tissue levels in the future. Continued sampling and analysis is essential to validate the assumption that accumulation of PBDEs will slow in the future.

Mean ΣDDT levels in the blubber of J, K, and L pods vary from 37,000 ± 39,000 ng/g lw, 72,000 ± 64,000 ng/g lw, and 61,000 ± 51,000 ng/g lw, respectively. They also range from 1,200 ng/g lw in a J pod individual to 210,000 ng/g lw in an L pod individual (Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). In fact, juvenile killer whales in K pod had more than double the levels of ΣDDTs than did individuals in J pod (Krahn et al. 2009). These higher ΣDDT levels in K and L pod members are likely because of the higher regional input of DDTs in California (Eganhouse et al. 2000, Bay et al. 2003) and the observation that both K and L pods travel to forage on prey (i.e., Chinook salmon) that originate in the Central Valley and remain primarily on the continental shelf south of Cape Blanco during their adult life stage (Krahn et al. 2009). In contrast, J pod has not yet been observed in California waters and is more frequently observed in the inland waters of Washington and British Columbia (Hauser et al. 2007). Because both K and L pods travel to forage on prey in California, their ΣDDTs/ΣPCBs ratios did not significantly differ from each other, but were significantly higher than those observed for J pod (Krahn et al. 2007a, Krahn et al. 2009, NWFSC unpubl. data). Therefore, the higher regional input of ΣDDTs from California is reflected in the higher contaminant ratios in K and L pod members. Like the pattern found for PCBs, ΣDDT body burdens in Southern Resident killer whales are substantially higher than other resident killer whale populations and significantly lower than the transient or offshore populations (Krahn et al. 2007b).

In summary, although there is a potential trend toward declining blubber PCB concentrations in Southern Resident killer whales, the absolute values are still very high and probably harmful. PBDE concentrations in the blubber of Southern Resident killer whales have not yet shown the declining trend observed in other marine mammal species. Due to reductions in the production and use of PBDEs, tissue concentrations are anticipated to decrease in the future. DDT concentrations appear to be a signature of foraging in California waters. Tissue concentrations remain high in Southern Resident killer whales and other marine mammals that feed near California. The high levels of PCBs, PBDEs, and DDTs in Southern Resident killer whales have health implications that are influenced by interactions with other stressors, including the abundance of Chinook salmon, the vulnerability of certain life stages, and the interactive effects of contaminant mixtures. While we do not discuss health implications to transient killer whales, which generally have higher POP levels than resident killer whales, we would expect these stressors (abundance of prey, vulnerability of certain life stages, and interactive effects of POPs) to have similar effects on transient killer whale health. Although there are limited data to indicate if the Southern Resident or transient killer whales are experiencing adverse health effects from high POP exposure, the high levels of PCBs, PBDEs, and DDTs in these killer whales are a significant concern. Below we discuss the implications for the health of Southern Resident killer whales from exposure to these POPs.

Contaminated Prey and Nutritional Limitation

Based on contaminant levels in Pacific salmon throughout their geographic range and the contribution of Chinook salmon to the summer diet of killer whales, these fish are likely an important source of contaminants to Southern Residents. The elevated POP levels in Chinook salmon, particularly in populations from Puget Sound and Harrison Lake (Fraser River), have implications for the viability of Southern Resident killer whales that feed upon them. Based on modeled PCB values for whales, Hickie et al. (2007) concluded that a diet at the tissue residue guideline of 50 ng/g to protect human health, which is similar to the average concentration in Chinook salmon from Puget Sound and Harrison Lake (Fraser River), would place over 95% of the killer whale population above the PCB effects threshold for immunosuppression found in harbor seals (17,000 ng/g lw, Ross et al. 1996).

The levels of POPs in Chinook salmon populations may also be high enough to negatively impact the health of the fish and, indirectly, the whales' food supply. Approximately 22% of maturing and sub-adult Chinook salmon samples collected from Puget Sound had PCB concentrations above an effects threshold identified for salmonids (O'Neill and West 2009), which could potentially affect their abundance. Previous studies have demonstrated that juvenile Chinook salmon from more polluted waters in Puget Sound have an increased risk of immunosuppression and are more susceptible to disease than Chinook salmon in less polluted areas (Varanasi et al. 1993a, Arkoosh et al.1998), linking elevated contaminant levels with reduced health and survival (Arkoosh and Collier 2002).

Abundance of Chinook salmon along the west coast of North America has been correlated with survival and fecundity of resident killer whales (Ford et al. 2005, Ward et al. 2009, Ford et al. 2010, Ward et al. 2013, Vélez-Espino et al. 2014b). In the 1990s, concurrent declines occurred

in both the Southern Resident killer whales and Chinook salmon, suggesting the potential for nutritional stress in the whales (Ford et al. 2005, Ford et al. 2010). Food availability is strongly associated with lipid content in individual marine mammals (Aguilar 1987). When lipids in the blubber are mobilized because of nutritional limitation, the contaminants can become mobilized into circulation (Aguilar 1987, Krahn et al. 2002, Debier et al. 2006, Louis et al. 2014), where they have the ability to induce a toxic response. Thus, nutritional stress from reduced Chinook salmon populations may act synergistically with high contaminant burdens in Southern Resident killer whales and result in higher mortality rates, reduced fecundity, or other contaminant-induced adverse health effects.

Unlike with Southern Residents that have experienced population declines, the West Coast transient killer whale population, which ranges from California to southeastern Alaska (Allen and Angliss 2014), appears to have grown rapidly from the mid-1970s to the mid-1990s, likely due to increased survival and fecundity as well as increased immigration of animals to the study area (DFO 2009). Their primary prey, harbor seals, also experienced rapid growth during this time (DFO 2009). Since 2008, transient killer whale mortality has been low and recruitment has been high (Towers et al. 2012). Transient killer whales have substantially higher contaminant burdens (Ross et al. 2000), but they do not appear to be experiencing nutritional stress or adverse health effects. The extent of influence that nutritional stress has on POP exposure and adverse health effects is currently unknown.

Endocrine Disruption and Sensitive Life Stages

Certain life stages are particularly vulnerable to endocrine disruption, such as thyroid hormone disruption. For example, because PCBs and PBDEs can transfer across the placenta and blood–brain barriers, the fetus (including the fetal brain) can be exposed to relatively high contaminant loads (Meerts et al. 2000). This influx of toxicants at such a young age is troubling because the growth and development of an individual are highly dependent on normal levels of thyroid hormones (Boas et al. 2006). Contaminants may disrupt normal hormone function (e.g., Hall et al. 2003) and thereby interfere with these developmental processes (Eriksson et al. 2002, Eriksson et al. 2006). The metabolism of a younger whale may also not be fully developed, hampering biotransformation and elimination of these pollutants. As a result, killer whale calves may be intrinsically more susceptible to contaminant-induced endocrine disruption because they are exposed to relatively high contaminant concentration levels, their capacity to eliminate these contaminants is reduced, and exposure occurs during critical stages of their development.

Endocrine disruption is difficult to observe in free-ranging killer whales, and the implications for individual and population health are unclear. Based on extrapolation from laboratory animal and human studies, the effects could include alterations to a calf's metabolism or growth and development. Altering these processes could affect the amount of prey required to meet energetic demands and also impair an animal's ability to metabolize other toxic chemicals. As a consequence, this may lead to further accumulation and intensify the sublethal and lethal effects of pollutants.

It has been well documented in the literature that exposure to endocrine disrupting chemicals during sensitive periods of gestation and development can impact an individual's reproductive health (see Fowler et al. 2012 for a review). Impacts may include delayed or premature physical or sexual maturity, reduced fecundity, or lack of reproductive success. However, killer whales have long life spans with variable recruitment, making it difficult to detect, assess, or interpret small changes in life history traits. A recent examination of the life history parameters and reproductive patterns in female resident killer whales from southern Alaska revealed that the mean age of first reproduction was 13 years (Matkin et al. 2014). In Northern and Southern Resident killer whales, the mean age at first reproduction is 14 to 15 years of age (Olesiuk et al. 2005), where "age at first reproduction" is defined by having conceived and produced a viable calf. Male resident killer whales in all three communities typically reach sexual maturity around 12 years of age and reach physical maturity by age 18 (Olesiuk et al. 2005, Matkin et al. 2014). Recently, Ward et al. (2009, 2013) estimated a regional difference in fecundity between the Southern Residents and the Northern Residents, and found that Southern Residents have lower production. Although the Southern Residents' and Northern Residents' geographic ranges overlap to some degree and both populations consume salmon as a primary prey species, regional differences appear to have translated to lower calving rates in the Southern Resident population. Vélez-Espino et al. (2014a) also found significantly lower fecundity and survival of viable calves in the Southern Resident population compared to the Northern Resident population. It is unclear as to why a disparity in fecundity and calf survival exists between these two populations, or why the age at maturity differs between the three resident communities. It is notable that the Northern Residents have significantly lower levels of some POPs that affect reproductive performance than the Southern Residents. Further, Alaska residents sampled off the Aleutian Islands have lower POP levels than Northern Residents (Table 3).

Exposure to endocrine disrupters at a young age may also affect fetal and perinatal survival. However, it is unknown if POP exposure in the Southern Resident population has caused an increase in neonatal or calf mortality. Because of the seasonality of the births, calf mortality is often missed, and estimated calving intervals are likely minimum estimates of calf production. In resident killer whales, calving intervals range from 2–14 years and significantly increase with advancing age (Olesiuk et al. 2005, Matkin et al. 2014). For example, in Alaska residents, the average calving interval for a 20-year-old was estimated at 4.3 years, whereas by age 40, the estimated average calving interval was 6.5 years (Matkin et al. 2014). An increase in the calving interval may indicate the onset of senescence, calf mortality, or another form of reproductive failure. In an early study, Olesiuk et al. (1990) calculated that viable calving intervals were marginally (but significantly) different between the more contaminated Southern Resident population (mean of 5.86 years) and the less contaminated Northern Resident population (mean of 5.02 years). Subsequently, Olesiuk et al. (2005) further refined the calving interval in the Northern Resident population during periods of population growth (mean of 4.88 years) and during periods of stability or no growth (mean calving interval of 5.53 years). Although the calving intervals vary and are only estimates, a reduction in the production of viable calves may indicate a reduction in fecundity or pregnancy rates that can impact the status and growth of the population.

Endocrine disruption may also lead to sex hormone alterations, which can sometimes result in permanent reproductive impairment. Altering sex steroids in males and females may result in premature births, reduced pregnancy rates, or generally reduced or abrogated fertility in females.

As with other mammalian species (e.g., Hany et al. 1999, Kuriyama et al. 2005), male killer whales exposed to contaminants may exhibit reductions in sperm and spermatid counts, testicular weights, and circulating testosterone levels. Any reduction in fecundity in Southern Residents will only further reduce the population size and recruitment rate. This potential to affect the population growth of a restricted population highlights the severity of the issue.

Contaminant exposure during critical periods of neurodevelopment can also cause significant changes in behavior and cognitive impairment in several species (e.g., Eriksson et al. 2001, Viberg et al. 2003, Viberg et al. 2006, Costa and Giordano 2007, Johansson et al. 2008). Reduced motor and brain function have been associated with exposure to endocrine disruptors in laboratory species (e.g., Dingemans et al. 2011, Kiciński et al. 2012), and, based on extrapolation to killer whales, such exposure could have significant impacts on behavior and survival. For example, killer whales rely to some degree on memory for foraging success. Reduced learning or impaired memory may affect their ability to find and capture prey and interact with other pod members. As killer whale calves mature, they likely develop their call repertoires through learning from closely associated individuals (see references within NMFS 2008). Because killer whales are in highly stable social groups, learning and remembering complex vocalizations are necessary for the well-being of the individuals and the group.

Immunotoxicity and Disease Susceptibility

Although there are few studies of the effects of POPs at the parturition and perinatal stages, the impacts of exposure are important. Northern fur seal (*Callorhinus ursinus*) pups that ingest POPs through nursing displayed a significant reduction in antibody response with higher contaminant exposure levels, indicating a greater predisposition for opportunistic or secondary infections (Beckmen et al. 2003). A comparison of in vitro peripheral blood immune function between seal pups residing in more (Baltic Sea) or less (Atlantic Ocean) polluted locations found a strong negative correlation between levels of blubber PCBs and mitogen-induced lymphoproliferation (Sørmo et al. 2009); a similar effect was observed in adult seals (de Swart et al. 1994, de Swart et al. 1995). This type of immune compromise in neonates bodes poorly for the overall health status and well-being of animals, and for their anticipated life expectancies.

Infectious diseases are a major source of morbidity and mortality for marine mammals of all ages. Between 2000 and 2006, 36.6% of marine mammal stranding mortalities in Cape Cod, Massachusetts were attributed to infectious disease (Bogomolni et al. 2010). From 1992 to 2001 along the central California coast, infectious diseases were the primary cause of death for 34.2% (437 of 1,277) of the live stranded northern elephant seals and 30.6% (288 of 940) of the live stranded harbor seals (Colegrove et al. 2005). Clearly, infectious diseases are a substantial contributor to marine mammal morbidity and mortality. For killer whales, pathogen exposure may occur through ingestion of pathogens, vectors, or intermediate host species; via inhalation of aerosolized pathogens; or by aspiration of the sea surface microlayer. Many of these recognized etiologic agents (Appendices A through C) are found exclusively within the marine environment or are terrestrially sourced. In many stranded killer whales, commensal flora appear to overwhelm or take advantage of compromised immune or inflammatory systems,

resulting in a proximate cause of death of septicemia or bacteremia.[3] For Southern Resident killer whales in particular, adverse health effects from contaminant exposure may be exacerbated by prey reduction and increased environmental pathogen contamination, culminating in increased susceptibility, morbidity, and mortality from multiple stressors (Sih et al. 2004).

At present, the range of pathogens or infectious diseases reported for killer whales is relatively small compared to more abundant, related, or sympatric odontocetes. The lack of defined pathogens may be due to reduced availability of live animals (wild or captive) and carcasses for diagnostic examination. Only 4–5% and 10% of dead Southern and Northern residents, respectively, have been available for necropsy, and from 1980–2004, few complete necropsies were performed. Nonetheless, researchers have made efforts to catalog and assess the threat of infectious diseases (Van Bressem et al. 1999, Gaydos et al. 2004), and a necropsy manual specifically for killer whales has been compiled, including tests for disease pathogens (Raverty et al. 2014). Appendix A presents pathogens and infections reported for wild and public display killer whales that were identified by culture, molecular screening, histopathology, or antibody detection. In some cases, infection was not necessarily associated with inflammation, but was suggestive of asymptomatic carriers (*Brucella* and *Salmonella*; Raverty et al. 2004 and Colegrove et al. 2010, respectively).

Interactive Effects

The pharmacodynamic and pharmacokinetic characteristics (i.e., what the contaminants do to the body and what the body does to contaminants, respectively) of PCBs and PBDEs can be species-specific, and influence the sensitivity in a species to toxic exposure. Thus, caution must be used when extrapolating risks between species (Schwacke et al. 2002). However, it is likely that killer whales are susceptible to synergistic effects of these two persistent pollutants, likely potentiating in vivo toxic health effects of individual contaminants.

Based on available evidence to date, killer whales assimilate multiple contaminants from prey and the environment, which likely interact and may enhance toxic effects. Historically, single class compound interactions appear to have been characterized, and only in more recent years have studies examined the health impacts of exposure to POP mixtures. Killer whales are exposed to a variety of chemicals, some of which may interact synergistically and cause enhanced toxicity in the whales. It is difficult to predict health effects in these whales from these mixture interactions, but disregarding mixture effects may underestimate risk to an individual or to the population.

For chemicals that likely interact additively (e.g., dioxin-like PCBs), the most accepted approach to determine the toxicity has been to use toxic equivalency factors (TEFs), which assume an additive effect and a common mechanism of action. The toxicity of dioxin-like PCBs is reported in relation to toxic equivalency quotients (TEQs) using TEFs. Calculations of the TEFs of specific PCB congeners are based on the biological response of the congener relative to the biological response to dioxin. In other words, the ability of PCBs to induce dioxin-like responses is

[3] Raverty, S. 2014. Pers. commun. Ministry of Agriculture and Lands, Animal Health Center, Abbotsford, BC, Canada.

expressed as a ratio. The TEQ of a mixture, considered to reflect the potential toxic impact more accurately, is calculated by the sum of the PCB congener concentrations in a mixture multiplied by their TEFs (O'Shea 1999). Silva et al. (2002) found this approach to be useful in determining the mixture effects of xenoestrogens when the mixture components had similar modes of action and similar concentration–response curves. However, this approach does not estimate the potential toxic impact from exposure to non-dioxin-like congeners. Non-dioxin-like chemicals may not act as AhR agonists in the way dioxin-like congeners do, and therefore may result in non-additive effects (i.e., synergistic or antagonistic). Thus, the TEQ approach, which adequately predicts effects for some chemicals, is likely the incorrect approach to use for mixtures containing both dioxin-like and non-dioxin-like chemicals.

Unlike the dioxin-like congeners that are more readily excreted, there is a selective retention of the non-dioxin-like congeners in marine mammals, likely because of a reduced capacity to metabolize some of these chemicals. For example, the non-planar congener, PCB-153, is not eliminated as efficiently as the dioxin-like congeners and is thus one of the most abundant congeners in both humans and wildlife. For this reason, it has often been used as a marker for total PCB burden (Fischer 2008). In general, the lower chlorinated PCBs and lower brominated PBDEs are less resistant to chemical biotransformation than the higher chlorinated or brominated congeners. However, metabolism appears to be enhanced at higher contaminant loads, and the ability of the individual to eliminate some contaminants from the body can improve with age (Aguilar 1987). Because the transformed by-product, or metabolite, may be more biologically active or more toxic than the parent compound (O'Shea 1999), it is important not only to adequately predict the toxicity of mixtures of the parent congeners, but also to establish the role of these metabolites on the health of killer whales.

The practice of examining only high doses of contaminants, especially endocrine disruptors, may also underestimate the risk to the killer whales. Endocrine disruptors have been shown to produce non-monotonic (or non-linear) dose–response effects. For example, Yordy et al. (2010a) observed a non-monotonic dose–response relationship for an estrogenic mixture containing biologically relevant concentrations of trans-nonachlor and 4,4'-DDE. At lower concentrations, the estrogenic response of the mixture in the absence of estradiol was additive; however, at higher concentrations, the estrogenic response was less than additive. Furthermore, the response was eliminated at the highest concentration (Yordy et al. 2010a). The non-monotonic dose–response relationship is not uncommon in the literature (see Vandenberg et al. 2012). Indeed, in a separate study, cell proliferation was observed when cells were treated with 17β-estradiol and low concentrations of PCB congeners -138, -153, and -180, whereas inhibited cell growth was observed at high concentrations of these PCB congeners (Bonefeld-Jørgensen et al. 2001).

Some contaminants can also interact at doses below the no-observed-effect concentrations (NOECs) and yet produce significant effects (Silva et al. 2002). For example, Crofton et al. (2005) tested the hypothesis that a mixture of thyroid hormone-disrupting chemicals has additive dose–response effects in female rats. The chemicals tested were dioxin, furan, and 11 PCB congeners. They found a significant departure from additivity. At the three higher doses, total T_4 was reduced more than predicted by the additive dose–response curve (i.e., the additivity model underestimates the observed toxic effects of the mixture). Thus, they demonstrated that the

effects from a mixture consisting of thyroid hormone disrupters can be additive at low doses and synergistic at high doses, and, more importantly, the highest mixture dose levels were at or below the NOECs of the chemicals. Therefore, even low concentrations of persistent pollutants, when combined together, may have the potential to cause adverse effects in the Southern Resident killer whales. The available data suggest that the health effects of persistent pollutants, such as PCBs, PBDEs, and DDTs, should not be considered in isolation, a critical point for risk assessment.

Data Needs and Future Directions

This report provides the most current knowledge about PCBs, PBDEs, and DDTs in Southern Resident killer whales and the implications for the whales' health from pollutant exposure. Although progress has been made in identifying potential effects from exposure to toxicants, there are uncertainties and data gaps that will need to be addressed in order to draw meaningful conclusions about the health of the killer whales. The past decade of research has shown that some of the most important threats facing the whales, such as high contaminant body burdens, cannot be addressed without a long-term commitment. The threat of contaminants is challenging, particularly considering that these long-lived whales remain contaminated by persistent pollutants that were banned decades ago. In this section, we describe the current data needs and recommend future directions. Although this discussion of data gaps is not exhaustive, we consider it to be an important first step.

Persistent Pollutants in Killer Whales

Researchers have measured PCBs, PBDEs, and DDTs in the blubber and scat of the majority of individuals in the Southern Resident killer whale population (Ross et al. 2000, Rayne et al. 2004, Krahn et al. 2007a, Krahn et al. 2009, Lundin et al. 2015, NWFSC unpubl. data). However, continued efforts to assess status and trends to understand the long-term health implications for these whales are necessary. In addition to the persistent pollutants that we focused on in this report, there are other toxic chemicals of concern on which future research should focus its efforts. For example, although PAH exposure has been documented previously in various species of marine mammals (Krahn et al. 1993, Holsbeek et al. 1999, Marsili et al. 2001, Leung et al. 2005, Goldstein et al. 2009, Fair et al. 2010, Moon et al. 2011), few PAH exposure data are available for cetaceans and pinnipeds from the Pacific Northwest. In general, relatively low concentrations (<100 ng/g ww) of these compounds have been measured in marine mammal tissues (e.g., blubber, liver, muscle), except in certain visibly oiled harbor seals and stranded cetaceans collected in Prince William Sound, Alaska, after the 1989 *Exxon Valdez* oil spill (Varanasi et al. 1993b, Frost et al. 1994), and in Indo-Pacific humpback dolphins (*Sousa chinensis*) from south China waters (Leung et al. 2005). Data on baseline exposure levels of parent and alkylated PAHs (e.g., in liver and/or feces) or PAH metabolites in other matrices (e.g., bile and/or feces) of Southern Resident killer whales are lacking, but are needed to determine if changes in PAH levels and/or patterns occur over time, and if these exposure levels are associated with biological effects. For example, in the aftermath of an oil spill in the region, baseline levels of PAHs could be compared to those measured in the tissues/fluids of Southern Resident killer whales post-spill to determine if increased PAH exposure had occurred.

Like the data on PAHs, baseline data on contemporary concentrations of toxic trace elements in the tissues of Southern Resident killer whales are scarce. The toxic non-essential elements most likely to pose a health risk to cetaceans are mercury, lead, and cadmium. Tissues of a few Southern Residents have been analyzed for trace metals (Wiles 2004). Levels of total mercury in skin biopsy samples of approximately 20 Southern Resident killer whales have been determined

(Anulacion et al. in prep.), but it is unknown how these concentrations compare to those of other tissues and any biological effects associated with skin biopsy levels. A previous review on the potential effects of toxic metals on killer whales from Washington State indicates that these compounds may pose a greater threat to their prey than to the whales (Wiles 2004).

In 2013, the Environmental Protection Agency, in coordination with NMFS, hosted a series of technical working groups aimed at discussing PBDEs in Puget Sound and their effects on Southern Resident killer whales. The group, consisting of scientists from federal and state governments, as well as from universities in the United States and Canada, provided their recommendations and highlighted data gaps and uncertainties (Gockel and Mongillo 2013). Although their focus was specifically on PBDEs, their recommendations apply to persistent pollutants in Southern Residents in general. Here we list the projects currently in progress, provide our recommendations for future work, and highlight some of the recommendations derived from the PBDE working groups:

- Collect baseline measurements for PAH and PAH metabolite levels.
- Measure levels of metals, including mercury, in the skin of Southern Resident killer whales.
- Combine multiple sources of data on individual killer whales in a common database, to allow for the development of individual health profiles.
- Continue to collect blubber and fecal samples from Southern Residents, to measure contaminant levels in combination with health indices (including hormone levels and immune response indices).
- Estimate the proportion of toxicants in fecal samples that is attributable to the whales' prey, and the proportion attributable to the whales' body burden.
- Evaluate the whales' health and nutritional status, and describe how these change both seasonally and between years.
- Create an integrated database of analyzed and archived samples.
- Archive tissues from stranded killer whale samples with the National Tissue Bank.
- Describe the congener-specific profiles of killer whales and their prey, to help reveal which compounds are more readily metabolized by the whales and therefore excreted, and which compounds more readily bioaccumulate and are therefore a greater health threat to the whales.

Persistent Pollutant Exposure in Calves

Some estimates of organochlorine transfer from cetacean mothers to calves have been made. Most of these studies have estimated POP transfer during gestation (e.g., Duinker and Hillebrand 1979, Tanabe et al. 1981, Tanabe et al. 1982, Aguilar and Borrell 1994, Borrell et al. 1995, Salata et al. 1995), while only a few have estimated transfer during lactation (e.g., Fukushima and Kawai 1981, Tanabe et al. 1981, Aguilar and Borrell 1994, Borrell et al. 1995). Studies that have attempted to quantify POP transfer during both gestation and lactation (e.g., Aguilar and Borrell 1994, Borrell et al. 1995) are rare. A wide variety of methods have been utilized, and more often than not,

studies rely on samples collected from deceased individuals and incorporate several assumptions to estimate transfer rates (e.g., Duinker and Hillebrand 1979, Fukushima and Kawai 1981, Tanabe et al. 1981, Tanabe et al. 1982, Cockcroft et al. 1989, Aguilar and Borrell 1994, Borrell et al. 1995, Kajiwara et al. 2008). Consequently, estimates are highly variable and may not be accurate.

Direct quantification of POP transfer during both gestation and lactation is necessary to adequately assess the risk of exposure for neonatal cetaceans. Furthermore, a description of contaminant transfer dynamics is also needed, to assess the risk of exposure for the reproductive females. However, to date, only two studies conducted on pinnipeds have directly quantified the dynamics of PCB transfer from mothers to pups or to the fetus, by sampling blubber, milk, and blood from live animals at several intervals over the lactation period and last trimester of pregnancy (e.g., Debier et al. 2003, Greig et al. 2007). Both cetaceans and pinnipeds have a blubber layer that serves as the energy source for milk production—and as a reservoir for POPs—yet their life history patterns and behavior during lactation differ substantially (e.g., most female pinnipeds undergo a fasting period during lactation, whereas delphinid females do not). Thus, contaminant transfer dynamics during lactation may differ between delphinids and pinnipeds. Results from the few studies that have investigated POP levels in live delphinids support this hypothesis. For example, Ridgway and Reddy (1995) reported that PCB and DDT levels in milk from captive bottlenose dolphins decreased over the course of lactation (>600 days), in contrast to the pattern observed over the 20-day lactation period of gray seals (Debier et al. 2003). Preliminary results from a study on PCB, PBDE, and DDT transfer from female bottlenose dolphins to their calves during lactation (>400 days) also suggest that POP levels in milk tend to decrease over the course of lactation, while levels in calf serum increase (Noren et al. unpubl. data). Although some data on contaminant transfer during lactation in live delphinids exist, additional studies are needed to better understand the dynamics of contaminant transfer in cetaceans. Such studies could:

- Collect blubber and/or blood samples from captive females at several intervals before and during pregnancy and from their calves (or stillborn calves) soon after birth, to quantify contaminant transfer and describe transfer dynamics in cetaceans during gestation.
- Collect milk samples (from captive mothers) and blubber and/or blood samples (from both captive mothers and calves) soon after birth and at several intervals during the nursing period, to quantify contaminant transfer and describe transfer dynamics in cetaceans during lactation.
- Examine neonatal exposure and subsequent effects on the developing immune system (de Guise et al. 2003).
- Establish and identify the POP levels in circulation at which health effects occur in killer whales, to help assess risk to both calves and pregnant or lactating females.

Persistent Pollutants in Prey and Prey Availability

Persistent pollutants and prey limitation are also important threats facing killer whales, but cannot be addressed without a long-term commitment. Recovery of threatened and endangered salmon, for example, is a monumental task in itself and is expected to take many years. Chemical tracer information can be used to infer the foraging habitats of salmon and their potential overlap with the Southern Residents. Historically, the Harrison population was the most abundant of all the Fraser Chinook salmon populations, but in recent years there have been lower returns (Henderson and Graham 1998). The Harrison population is believed to have a more localized marine distribution near the urbanized inland waters of Washington and British Columbia, and thus is anticipated to have higher contaminant concentrations relative to other Fraser Chinook salmon populations. Samples of Harrison Chinook salmon have been collected, but the tissues have not yet been analyzed. Additional information on the fat content and length of the fish (O'Neill et al. 2014) can also be used to calculate whether these fish or other fish populations represent a significant source of energy and lipophilic contaminants to the whales. Here we provide a list of projects currently in progress, and our recommendations for future research:

- Collect data on stable isotopes and POPs from spring-run Chinook salmon populations, especially those from the Columbia River.
- Collect data on POPs, stable isotopes, and lipids from additional Fraser River Chinook salmon populations, particularly the Harrison population.
- Collect stable isotope and POP data on other marine fish species, especially halibut, steelhead, and lingcod, from various populations along the west coast of North America, in order to help elucidate whether these species are significant prey to Southern Residents in the winter months.
- Continue to establish and identify the levels at which health effects occur in Chinook salmon.
- Reestablish monitoring efforts, to measure persistent pollutants in Chinook salmon from the outer coast and from the inland waters.
- Extend contaminants monitoring efforts to other fish, such as herring.

Health Effects

A major barrier to assessing health risks caused by POP exposure is poor information on dose–response relationships and genetic variability within populations of concern. However, there have been attempts to identify quantitative values that may be applied in risk assessment. For example, based on studies measuring tissue concentrations and effects of exposure to PCBs in harbor seal, European otter (*Lutra lutra*), and mink (*Neovison vison*), Kannan et al. (2000) determined the suggested threshold values for physiological effects in marine mammals for liver and blood (8.7 μg/g lw) and blubber (17 μg/g lw). POP levels in the blubber of Southern Resident killer whales frequently exceed this estimated effects threshold, particularly in males and juveniles (Krahn et al. 2007a, Krahn et al. 2009). A reconstruction of PCB histories for Northern and Southern Resident killer whale prey and concentrations in whale tissues was used to formulate cumulative population projections for body burdens (Hickie et al. 2007). Based on a comparison of those projections against the effects threshold of 17 μg/g lw, 96% of the Southern Resident population may continue

to bear burdens that exceed that threshold until 2020, and under a slower environmental turnover scenario, 5% of the population could still exceed the threshold by 2089 (Hickie et al. 2007). A risk assessment of PCB effects on reproductive success in three populations of bottlenose dolphins used gender- and age-matched measurements to derive a dose–response function. The median effective concentration (EC50) was calculated to be 33 μg/g lw for liver or blubber, and an approximate benchmark dose (an increasingly employed alternative to the no-observed-adverse-effect level, or NOAEL) was determined to be 14.8 μg/g lw (Schwacke et al. 2002). While this risk assessment was aimed at reproduction, not immune function, the proximity of the benchmark dose to the earlier physiologic threshold estimate suggests these values are physiologically relevant. To date, no risk assessment of infectious disease mortality associated with contaminant levels in tissues has been conducted for killer whales. A case–control risk study of harbor porpoise found a 2% increased risk of mortality because of infectious disease for each 1 mg/kg lw rise in blubber PCBs, with a doubling of risk occurring at 45 mg/kg lw (Hall et al. 2006a).

Although predictive models based on laboratory organisms are often applied to wild animals, extrapolation of these models to marine mammals can be incorrect (Mori et al. 2008). Support for an epidemiologic role for organic contaminants in marine mammal diseases has relied primarily on association or correlation patterns, rather than mechanistic data (Ross 2002). For example, the impact of tissue PCB and DDT contaminants was inferred in a comparison of two epizootics of morbillivirus in Mediterranean striped dolphin, where the earlier, more virulent epizootic occurred among animals with tenfold higher tissue concentrations of contaminants than the later, less-virulent outbreak (Castrillon et al. 2010). The increased levels of PCBs were hypothesized to have suppressed the immune system, increasing susceptibility to infection and ultimately leading to mortality (Aguilar and Borrell 1994). However, it remains unclear if the suppressed immune system was a direct effect from PCB exposure or if other indirect processes contributed to mortality.

Currently, the effects of chronic exposure to PCBs, PBDEs, and DDTs on Southern Resident killer whales are unknown. During times of lipid mobilization (e.g., nutritional stress, migration, pregnancy, or nursing), these pollutants enter into circulation and have the potential to cause deleterious health effects. However, the extent of pollutant mobilization is unclear, and the consequences from these spurts of exposure due to lipid mobilization are not known. The PCB, PBDE, and DDT levels that will cause deleterious health effects in the Southern Resident killer whales are also unknown, and threshold levels of health effects in other species may not be directly applicable to the killer whales. To date, threshold levels have been observed in other species for single contaminants, and these can inform killer whale analyses, but how threshold levels may change when interaction occurs in a mixture is unknown.

An emerging aspect of health assessment is the recognition that the commensal microbial community (the "microbiome") of animals represents a significant effector and indicator of changes in physiology, with the greatest attention on gut microbiomes. Analysis of bacterial DNA from fecal material characterizes the gastrointestinal microbial community structure, which can reflect changes in metabolic state and health (e.g., Kinross et al. 2011). Although fecal material from Southern Residents is already analyzed for hormones, prey sources, and individual animal identification, microbiome analysis has not been performed. Additionally, microbiomes associated with Southern Residents can be assessed for the presence of antibiotic resistance genes

as a measure of exposure to human influences (e.g., Penders et al. 2013), including wastewater treatment effluent and stormwater runoff. An evaluation of respiratory microbiota of Southern Residents found resistance to widely used veterinary and human antibiotics in more than half of the tested isolates, and multi-drug resistance was common (Schroeder et al. 2010, Raverty et al. in prep.). The expansion of clinically significant genes for antibiotic resistance is widely recognized as a signal of anthropogenic impact, and is considered a negative indicator of environmental quality (e.g., Perry and Wright 2013). Here we provide our recommendations for future research to fill identified data gaps:

- Conduct a risk assessment of infectious disease morbidity and mortality associated with contaminant levels in tissues for killer whales.
- Perform microbiome analyses complementary to the other health measures (e.g., from scat collection).
- Conduct other noninvasive sampling, such as the collection of exhaled breath spray and shed mucus, for pathogen and microbiome analyses.
- Assess for the presence of antibiotic resistance genes in tissues and microbiomes.
- Correlate health measurements and reproductive endpoints to contaminant levels (in blubber and blood), and develop other biomarkers of exposure and toxicity.

Conclusions

Southern Resident killer whales frequent marine waterways where relatively high levels of PCBs, PBDEs, and DDTs are found. Adverse effects from exposure to these persistent pollutants are known to impact reproduction, immune function, and neurodevelopment, and to disrupt the endocrine system, in multiple mammalian species. Exposure to a mixture of these contaminants can heighten these detrimental biological effects and may hinder recovery of the Southern Resident killer whale population. Understanding the factors that affect whales' health will help identify and prioritize the most important threats, understand how those threats may interact, and begin planning what we can do to reduce their impacts. We have provided recommendations to address some of the data gaps and guide management actions to advance the recovery of Southern Resident killer whales. Currently, new technologies are being developed to better understand disease threats, assess individual body condition and health of individuals, and gain a better understanding of the health effects of carrying large contaminant burdens. Ultimately, long-term monitoring, reducing exposure, and determining the risks posed by PCBs, PBDEs, and DDTs in the killer whales are essential for the effective protection of this endangered species.

References

Addison, R. F., and P. F. Brodie. 1987. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating grey seal *Halichoerus grypus*. Can. J. Fish. Aquat. Sci. 44:782–786.

ADEC (Alaska Department of Environmental Conservation). 2011. Fish monitoring program: Analysis of organic contaminants, 31 p. Alaska Department of Environmental Conservation.

Aguilar, A. 1987. Using organochlorine pollutants to discriminate marine mammal populations: A review and critique of the methods. Mar. Mamm. Sci. 3:242–262.

Aguilar, A., and A. Borrell. 1994. Reproductive transfer and variation of body load of organochlorine pollutants with age in fin whales (*Balaenoptera physalus*). Arch. Environ. Contam. Toxicol. 27:546–554.

Aguilar A., A. Borrell, and T. Pastor. 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. J. Cetacean Res. Manag. Special Issue 1:83–116.

Allen, B. M., and R. P. Angliss. 2014. Alaska marine mammal stock assessments, 2013. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-AFSC-277, 294 p.

Angell, C. M., J. Y. Wilson, M. J. Moore, and J. J. Stegeman. 2004. Cytochrome p450 1A1 expression in cetacean integument: Implications for detecting contaminant exposure and effects. Mar. Mamm. Sci. 20:554–566.

Anulacion, B. F., D. Boyd, M. B. Hanson, C. Emmons, R. W. Baird, G. S. Schorr, and G. M. Ylitalo. In prep. Total mercury levels in Pacific Northwest cetaceans. (Available from B. Anulacion, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle, WA 98112.)

Arkoosh, M. R., E. Casillas, P. Huffman, E. Clemons, J. Evered, J. E. Stein, and U. Varanasi. 1998. Increased susceptibility of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from a contaminated estuary to the pathogen *Vibrio anguillarum*. Trans. Am. Fish. Soc. 127:360–374.

Arkoosh, M. R., E. Clemons, P. Huffman, A. N. Kagley, E. Casillas, N. Adams, H. R. Sanborn, T. K. Collier, and J. E. Stein. 2001. Increased susceptibility of juvenile Chinook salmon to vibriosis after exposure to chlorinated and aromatic compounds found in contaminated urban estuaries. J. Aquat. Anim. Health. 13:257–268.

Arkoosh, M. R., and T. K. Collier. 2002. Ecological risk assessment paradigm for salmon: Analyzing immune function to evaluate risk. Hum. Ecol. Risk Assess. 8:265–276.

Atkinson, S., D. J. St. Aubin, and R. M. Ortiz. 2009. Endocrine systems. *In* W. E. Perrin, B. Wursig, and J. G. M. Thewissen (eds.), Encyclopedia of marine mammals, pp. 375–383. Academic Press, China.

Ayres, K. L. 2011. Distinguishing the impacts of prey availability and vessel traffic on an endangered killer whale (*Orcinus orca*) population. Thesis, 98 p. University of Washington, Seattle.

Ayres, K. L., R. K. Booth, J. A. Hempelmann, K. L. Koski, C. K. Emmons, R. W. Baird, K. Balcomb-Bartok, M. B. Hanson, M. J. Ford, and S. K. Wasser. 2012. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (*Orcinus orca*) population. PLoS ONE 7:1–12.

Bay, S. M., E. Y. Zeng, T. D. Lorenson, K. Tran, and C. Alexander. 2003. Temporal and spatial distributions of contaminants in sediments of Santa Monica Bay, California. Mar. Environ. Res. 56: 255–266.

Beacham, T. D. 1986. Type, quantity, and size of food of Pacific salmon (*Oncorhynchus*) in the Strait of Juan De Fuca, British Columbia. Fish. Bull. 84:77–89.

Beckmen, K. B., J. E. Blake, G. M. Ylitalo, J. L. Stott, and T. M. O'Hara. 2003. Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as a factor in free-ranging northern fur seal pups (*Callorhinus ursinus*). Mar. Pollut. Bull. 46:594–606.

Beineke, A., U. Siebert, M. McLachlan, R. Bruhn, K. Thron, K. Failing, G. Muller, and W. Baumgartner. 2005. Investigations of the potential influence of environmental contaminants on the thymus and spleen of harbor porpoises (*Phocoena phocoena*). Environ. Sci. Technol. 39:3933–3938.

Beineke, A., U. Siebert, G. Muller, and W. Baumgartner. 2007. Increased blood interleukin-10 mRNA levels in diseased free-ranging harbor porpoises (*Phocoena phocoena*). Vet. Immunol. Immunopathol. 115:100–106.

Beineke, A., U. Siebert, P. Wohlsein, and W. Baumgartner. 2010. Immunology of whales and dolphins. Vet. Immunol. Immunopathol. 133:81–94.

Beineke, A., U. Siebert, A. Wunschmann, J. L. Stott, I. Prengel, E. Kremmer, and W. Baumgartner. 2001. Immunohistochemical investigation of the cross-reactivity of selected cell markers from various species for characterization of lymphatic tissues in the harbour porpoise (*Phocoena phocoena*). J. Comp. Pathol. 125:311–317.

Béland, P., S. de Guise, C. Girard, A. Lagacé, D. Martineau, R. Michaud, D. C. G. Muir, R. J. Norstrom, É. Pelletier, S. Ray, and L. R. Shugart. 1993. Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. J. Great Lakes Res. 19:766–775.

Bergeron, J. M., D. Crews, and J. A. McLachlan. 1994. PCBs as environmental estrogens: Turtle sex determination as a biomarker of environmental contamination. Environ. Health Perspect. 102:780–781.

Biscoe, M. L., C. M. Mutero, and R. A. Kramer. 2005. Working Paper 95: Current policy and status of DDT use for malaria control in Ethiopia, Uganda, Kenya, and South Africa, 30 p. International Water Management Institute, Colombo, Sri Lanka.

Black, N., R. Ternullo, A. Schulman-Janiger, A. M. Hammers, and P. Stapp. 2001. Occurrence, behavior, and photo-identification of killer whales in Monterey Bay, California. *In* 14th biennial conference on the biology of marine mammals, p. 26. Society for Marine Mammals, Vancouver, BC, Canada.

Blais, J. M., R. W. Macdonald, D. Mackey, E. Webster, C. Harvey, and J. P. Smol. 2007. Biologically mediated transport of contaminants to aquatic systems. Environ. Sci. Technol. 41:1075–1084.

Blasius, M. E., and G. D. Goodmanlowe. 2008. Contaminants still high in top-level carnivores in the Southern California Bight: Levels of DDT and PCBs in resident and transient pinnipeds. Mar. Pollut. Bull. 56:1973–1982.

Boas, M., U. Feldt-Rasmussen, N. E. Skakkebæk, and K. M. Main. 2006. Environmental chemicals and thyroid function. Eur. J. Endocrinol. 154:599–611.

Bogomolni, A. L., K. R. Pugliares, S. M. Sharp, K. Patchett, C. T. Harry, J. M. LaRocque, K. M. Touhey, and M. Moore. 2010. Mortality trends of stranded marine mammals on Cape Cod and southeastern Massachusetts, USA, 2000 to 2006. Dis. Aquat. Org. 88:143–155.

Bonefeld-Jørgensen, E. C., H. R. Andersen, T. H. Rasmussen, and A. M. Vinggaard. 2001. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158:141–153.

Bonefeld-Jørgensen, E. C., M. Long, R. Bossi, P. Ayotte, G. Asmund, T. Kruger, M. Ghisari, G. Mulvad, P. Kern, P. Nzulumiki, and E. Dewailly. 2011. Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: A case control study. Environ. Health 10:88.

Borrell, A., D. Bloch, and G. Desportes. 1995. Age trends and reproductive transfer of organochlorine compounds in long-finned pilot whales from the Faroe Islands. Environ. Pollut. 88:283–292.

Bowers, W. J., J. S. Nakai, I. Chu, M. G. Wade, D. Moir, A. Yagminas, S. Gill, O. Pulido, and R. Meuller. 2004. Early developmental neurotoxicity of a PCB/organochlorine mixture in rodents after gestational and lactational exposure. Toxicol. Sci. 77:51–62.

Boyd, I. L., C. Lockyer, and H. D. Marsh. 1999. Reproduction in marine mammals. *In* J. E. Reynolds and S. A. Rommel (eds.), Biology of marine mammals, pp. 218–286. Smithsonian Institution Press, Washington and London.

Breivik, K., A. Sweetman, J. M. Pacyna, and K. C. Jones. 2002. Towards a global historical emission inventory for selected PCB congeners—a mass balance approach. 1. Global production and consumption. Sci. Total. Environ. 290:181–198.

Brett, J. R. 1995. Energetics. *In* C. Groot, L. Margolis, and W. C. Clark (eds.), Physiological ecology of Pacific salmon, pp. 3–68. Univ. British Columbia Press, Vancouver, BC, Canada.

Brouwer, A., H. Hakansson, and A. Kukler. 1989. Marked alterations in retinoid homeostasis of Sprague-Dawley rats induced by a single i.p. dose of 10 μg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology 58:267–283.

Brown, D. W., B. B. McCain, B. H. Horness, C. A. Sloan, K. L. Tilbury, S. M. Pierce, D. G. Burrows, S-L. Chan, J. T. Landahl, and M. M. Krahn. 1998. Status, correlations, and temporal trends of chemical contaminants in fish and sediment from selected sites on the Pacific Coast of the USA. Mar. Pollut. Bull. 37(1–2):67–85.

Buchanan, D. L., S. Ohsako, C. Tohyama, P. S. Cooke, and T. Iguchi. 2002. Dioxin inhibition of estrogen-induced mouse uterine epithelial mitogenesis involves changes in cyclin and transforming growth factor-beta expression. Toxicol. Sci. 66:62–68.

Buchanan, D. L., T. Sato, R. E. Peterson,and P. S. Cooke. 2000. Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mouse uterus: Critical role of the aryl hydrocarbon receptor in stromal tissue. Toxicol. Sci. 57:302–311.

Buckman, A. H., N. Veldhoen, G. Ellis, J. K. Ford, C. C. Helbing, and P. S. Ross. 2011. PCB-associated changes in mRNA expression in killer whales (*Orcinus orca*) from the NE Pacific Ocean. Environ. Sci. Technol. 45:10194–10202.

Bull, J.C., P. D. Jepson, R. K. Ssuna, R. Deaville, C. R. Allchin, R. J. Law, and A. Fenton. 2006. The relationship between polychlorinated biphenyls in blubber and levels of nematode infestations in harbour porpoises, *Phocoena phocoena*. Parasitology 132:565–573.

Calambokidis, J. C., S. J. Jeffries, P. S. Ross, and M. G. Ikonomou. 1999. Final report: Temporal trends in contaminants in Puget Sound harbor seals. U.S. Environmental Protection Agency and Puget Sound Water Quality Action Team, Olympia and Tacoma, Washington, and Sidney, BC, Canada.

Calambokidis, J., S. Jeffries, P. S. Ross, and M. Ikonomou. 2001. Temporal trends in Puget Sound harbor seals. *In* Puget Sound research conference, 9 p. Puget Sound Water Quality Action Team, Seattle, WA.

Calambokidis, J., J. Peard, G. Steiger, J. Cubbage, R. L. DeLong. 1984. Chemical contaminants in marine mammals from Washington State. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-F/NWC-6, 167 p.

Carls, M. G., and J. P. Meador. 2009. A perspective on the toxicity of petrogenic PAHs to developing fish embryos related to environmental chemistry. Hum. Ecol. Risk Assess. 15:1084–1098.

Castrillon, J., E. Gomez-Campos, A. Aguilar, L. Berdie, and A. Borrell. 2010. PCB and DDT levels do not appear to have enhanced the mortality of striped dolphins (*Stenella coeruleoalba*) in the 2007 Mediterranean epizootic. Chemosphere 81:459–463.

Chamberlin, J. W., T. E. Essington, J. W. Ferguson, and T. P. Quinn. 2011. The influence of hatchery rearing practices on salmon migratory behavior: Is the tendency of Chinook salmon to remain within Puget Sound affected by size and date of release? Trans. Am. Fish. Soc. 140:1398–1408.

Cockcroft, V. G., A. C. De Kock, D. A. Lord, and G. J. B. Ross. 1989. Organochlorines in bottlenose dolphins *Tursiops truncatus* from the east coast of South Africa. Afr. J. Mar. Sci. 8:207–217.

Colegrove, K. M., D. J. Greig, and F. M. D. Gulland. 2005. Causes of live strandings of northern elephant seals (*Mirounga angustirostris*) and Pacific harbor seals (*Phoca vitulina*) along the central California coast, 1992–2001. Aquat. Mamm. 31:1.

Colegrove, K. M., J. A. St. Leger, S. Raverty, S. Jang, M. Berman-Kowalewski, and J. K. Gaydos. 2010. Salmonella Newport omphaloarteritis in a stranded killer whale (*Orcinus orca*) neonate. J. Wildl. Dis. 46:1300–1304.

Colegrove, K. M., S. Venn-Watson, J. Litz, M. J. Kinsel, K. A. Terio, E. Fougeres, R. Ewing, D. A. Pabst, W. A. McLellan, S. Raverty, J. Saliki, S. Fire, G. Rappucci, S. Bowen-Stevens, L. Noble, A. Costidis, M. Barbieri, C. Field, S. Smith, R. H. Carmichael, C. Chevis, W. Hatchett, D. Shannon, M. Tumlin, G. Lovewell, W. McFee, and T. K. Rowles. 2016. Fetal distress and in utero pneumonia in perinatal dolphins during the Northern Gulf of Mexico unusual mortality event. Dis. Aquat. Org. 119:1–16.

Collier, T. K., S. M. O'Neill, J. E. West, and N. L. Scholz. 2007. Toxic chemical contaminants and Puget Sound. Paper presented at Puget Sound Georgia Basin research conference, 26–29 March 2007, Vancouver, BC, Canada.

Costa, L. G., and G. Giordano. 2007. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. Neurotoxicology 28:1047–1067.

Crofton, K. M. 2004. Developmental disruption of thyroid hormone: Correlations with hearing dysfunction in rats. Risk Anal. 24:1665–1671.

Crofton, K. M., E. S. Craft, J. M. Hedge, C. Gennings, J. E. Simmons, R. A. Carchman, W. H. Carter, and M. J. DeVito. 2005. Thyroid hormone-disrupting chemicals: Evidence for dose-dependent additivity or synergism. Environ. Health Perspect. 113:1549–1554.

Cullon, D. L., M. B. Yunker, C. Alleyne, N. J. Dangerfield, S. O'Neill, M. J. Whiticar, and P. S. Ross. 2009. Persistent organic pollutants in Chinook salmon (*Oncorhynchus tshawytscha*): Implications for resident killer whales of British Columbia and adjacent waters. Environ. Toxicol. Chem. 28:148–161.

Dabin, W., F. Cossais, G. Pierce, and V. Ridoux. 2008. Do ovarian scars persist with age in all cetaceans: New insight from the short-beaked common dolphin (*Delphinus delphis Linnaeus*, 1758). Mar. Biol. 156:127–139.

Dahlman, H. J., P. I. Gaarder, M. Lovik, J. Alexander, G. Becher, and A. Bergman. 1994. PCB congeners and the murine immune system: The capacity of isolated congeners to decrease spleen-specific antibody-forming cell numbers varies with the toxic equivalence factors of the congeners. Toxicol. In Vitro 8:1033–1035.

Darnerud, P. O. 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environ. Int. 29:841–853.

Darnerud, P. O. 2008. Brominated flame retardants as possible endocrine disrupters. Int. J. Androl. 31:152–160.

Das, K., U. Siebert, A. Gillet, A. Dupont, C. Di-Poi, S. Fonfara, G. Mazzucchelli, E. De Pauw, and M. C. De Pauw-Gillet. 2008. Mercury immune toxicity in harbour seals: Links to in vitro toxicity. Environ. Health. 7:52.

Das, K., D. Virginie, P. Stéphane, and B. Jean-Marie. 2003. Heavy metals in marine mammals. *In* J. V. Vos, G. D. Bossart, M. Fournier, and T. O'Shea (eds.), Toxicology of marine mammals, pp. 135–167. Taylor and Francis, Washington, D.C.

Davison, A. N., and J. Dobbing. 1968. The developing brain. Appl. Neurochem. 178:178–221.

Debier, C., C. Chalon, B. J. Le Boeuf, T. de Tillesse, Y. Larondelle, and J. P. Thomé. 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (*Mirounga angustirostris*) during the post-weaning fast. Aquat. Toxicol. 80:149–157.

Debier, C., P. P. Pomeroy, C. Dupont, C. Joiris, V. Comblin, E. Le Boulengé, Y. Larondelle, and J-P. Thomé. 2003. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*. Mar. Ecol. Prog. Ser. 247:237–248.

de Boer, J., K. de Boer, and J. P. Boon. 2000. Toxic effects of brominated flame retardants in man and wildlife. Eviron. Int. 29:841–853.

de Boer, J., P. G. Wester, H. J. C. Klamer, W. E. Lewis, and J. P. Boon. 1998. Do flame retardants threaten ocean life? Nature 394:28–29.

deBruyn, A. M. H., M. G. Ikonomou, and F. A. P. C. Gobas. 2004. Magnification and toxicity of PCBs, PCDDs, and PCDFs in upriver-migrating Pacific salmon. Environ. Sci. Technol. 38:6217–6224.

DFO (Dept. Fisheries and Oceans Canada). 1999. Fraser River Chinook salmon. DFO science stock status report D6-11. Nanaimo, BC, Canada.

DFO (Dept. Fisheries and Oceans Canada). 2009. Recovery potential assessment for west coast transient killer whales. Canadian science advisory secretariat science advisory report 2009/039. Nanaimo, BC, Canada.

de Guise, S., K. B. Beckmen, and S. D. Holladay. 2003. Contaminants and marine mammal immunotoxicology and pathology. *In* J. G. Vos, G. D. Bossart, M. Fournier, and T. J. O'Shea (eds), Toxicology of marine mammals. New perspectives: Toxciology and the environment, pp. 38–54. Taylor and Francis, London.

de Guise, S., P. S. Ross, A. D. Osterhaus, D. Martineau, P. Beland, and M. Fournier. 1997. Immune functions in beluga whales (*Delphinapterus leucas*): Evaluation of natural killer cell activity. Vet. Immunol. Immunopathol. 58:345–354.

Desforges, J. P., P. S. Ross, and L. L. Loseto. 2012. Transplacental transfer of polychlorinated biphenyls and polybrominated diphenyl ethers in arctic beluga whales (*Delphinapterus leucas*). Environ. Toxicol. Chem. 31:296–300.

de Swart, R. L., P. S. Ross, H. H. Timmerman, H. W. Vos, P. J. H. Reijnders, J. G. Vos, and A. D. M. E. Osterhaus. 1995. Impaired cellular immune-response in harbour seals (*Phoca vitulina*) feeding on environmentally contaminated herring. Clin. Exp. Immunol. 101:480–486.

de Swart, R. L., P. S. Ross, L. J. Vedder, H. H. Timmerman, S. Heisterkamp, H. Vanloveren, J.G. Vos, P. J. H. Reijnders, and A. D. M. E. Osterhaus. 1994. Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. Ambio 23:155–159.

de Swart, R. L., P. S. Ross, J. G. Vos, and A.Osterhaus. 1996. Impaired immunity in habour seals (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: Review of long-term feeding study. Environ. Health Perspect. 104:823–828.

de Wit, C. A. 2002. An overview of brominated flame retardants in the environment. Chemosphere 46:583–624.

Dingemans, M. M., M. van den Berg, and R. H. Westerink. 2011. Neurotoxicity of brominated flame retardants: (In)direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. Environ. Health Perspect. 119:900–907.

Donkin, P., S. V. Mann, and E. I. Hamilton. 1981. Polychlorinated biphenyl, DDT, and Dieldrin residues in grey seal (*Halichoerus grypus*) males, females and mother–foetus pairs sampled at the Farne Islands, England, during the breeding season. Sci. Total Environ. 19:121–142.

Duinker, J. C., and M. Th. J. Hillebrand. 1979. Mobilization of organochlorines from female lipid tissue and transplacental transfer to fetus in a harbor porpoise (P*hocoena phocoena*) in a contaminated area. Bull. Environ. Contam. Toxicol. 23:728–732.

Ecology (Washington Department of Ecology) and Health (Washington Department of Health). 2006. Washington State polybrominated diphenyl ether (PBDE) chemical action plan: Final plan. Department of Ecology Publications Distributions Office, Olympia, Washington. No. 05-07-048.

Eganhouse, R. P., J. Pontolillo, and T. J. Leiker. 2000. Diagenetic fate of organic contaminants on the Palos Verdes Shelf, California. Mar. Chem. 70:289–315.

Egawa, H., Y. Inomata, S. Uemoto, K. Asonuma, T. Kiuchi, S. Fujita, M. Hayashi, M. A. Matamoros, K. Itou, and K. Tanaka. 2001. Biliary anastomotic complications in 400 living related liver transplantations. World J. Surg. 25:1300–1307.

Elfes, C. T., G. R. VanBlaricom, D. Boyd, J. Calambokidis, P. J. Clapham, R. W. Pearce, J. Robbins, J. C. Salinas, J. M. Straley, P. R. Wade, and M. M. Krahn. 2010. Geographic variation of persistent organic pollutant levels in humpback whale (*Megaptera novaeangliae*) feeding areas of the North Pacific and North Atlantic. Environ. Toxicol. Chem. 29:824–834.

Elliott, J. E., L. K. Wilson, and B. Wakeford. 2005. Polybrominated diphenyl ether trends in eggs of marine and freshwater birds from British Columbia, Canada, 1979–2002. Environ. Sci. Technol. 39:5584–5591.

El-Zein, R. A., D. A. Hastings-Smith, M. M. Ammenheuser, M. Treinen-Moslen, F. M. Gulland, and J. B. Ward. 2006. Evaluation of two different biomarkers for use in the assessment of toxic chemical exposure in California sea lions (*Zalophus californianus*). Mar. Pollut. Bull. 52:108–113.

EPA (Environmental Protection Agency). 1972. DDT ban takes effect. EPA press release, 31 December 1972.

EPA (Environmental Protection Agency). 2002. Columbia River Basin fish contaminant survey, 1996–1998. Report no. 910-R-02-006.

Eriksson, P., C. Fischer, and A. Fredriksson. 2006. Polybrominated diphenyl ethers, a group of brominated flame retardants, can interact with polychlorinated biphenyls in enhancing developmental neurobehavioral defects. Toxicol. Sci. 94:302–9.

Eriksson, P., E. Jakobsson, and A. Fredriksson. 2001. Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? Environ. Health Perspect. 109:903–908.

Eriksson, P., H. Viberg, E. Jakobsson, U. Orn, U. and A. Fredriksson. 2002. A brominated flame retardant, 2,2',4,4',5-pentabromodiphenyl ether: Uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. Toxicol. Sci. 67:98–103.

Erikstad, K. E., T. Moum, J. O. Bustnes, T. K. Reiertsen. 2011. High levels of organochlorines may affect hatching sex ratio and hatchling body mass in arctic glaucous gulls. Funct. Ecol. 25:289–296.

Ewald, G., P. Larsson, H. Linge, L. Okla, and N. Szarzi. 1998. Biotransport of organic pollutants to an inland Alaska lake by migrating sockeye salmon (*Oncorhynchus nerka*). Arctic 51:40–47.

Fair, P. A., J. Adams, G. Mitchum, T. C. Hulsey, J. S. Reif, M. Houdi, D. Muir, E. Wirth, D. Wetzel, E. Zolman,W. McFee, and G. D. Bossart. 2010. Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncates*) from two southeastern U.S. estuarine areas: Concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. Sci. Total Environ. 408:1577–1597.

Fair, P. A., E. Montie, L. Balthis, J. S. Reif, and G. D. Bossart. 2011. Influences of biological variables and geographic location on circulating concentrations of thyroid hormones in wild bottlenose dolphins (*Tursiops truncatus*). Gen. Comp. Endocrinol. 174:184–194.

Felleman, F. L., J. R. Heimlich-Boran, and R. W. Osborne. 1991. The feeding ecology of killer whales (*Orcinus orca*) in the Pacific Northwest. *In* K. Pryor and K. Norris (eds.), Dolphin societies: Discoveries and puzzles, pp. 113–147. Univ. California Press, Berkeley.

Fernie, K. J., J. L. Shutt, R. J. Letcher, I. J. Ritchie, and D. M. Bird. 2009. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. Environ. Sci. Technol. 43:2124–2130.

Fernie, K. J., J. L. Shutt, G. Mayne, D. Hoffman, R. J. Letcher, K. G. Drouillard, I. J. Ritchie. 2005. Exposure to polybrominated diphenyl ethers (PBDEs): Changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). Toxicol. Sci. 88:375–383.

Fischer, C. 2008. Developmental neurotoxicity in mice neonatally co-exposed to environmental agents. PCB, PBDE, methyl mercury and ionized radiation—interactions and effects. Acta universitatis Upsaliensis. Digital comprehensive summaries of Uppsala dissertations from the Faculty of Science and Technology 387, p. 76. Uppsala, Sweden.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008a. Neonatal co-exposure to low doses of an ortho-PCB (PCB 153) and methyl mercury exacerbate defective developmental neurobehavior in mice. Toxicology 244:157–165.

Fischer, C., A. Fredriksson, and P. Eriksson. 2008b. Coexposure of neonatal mice to a flame retardant PBDE 99 (2,2',4,4',5-pentabromodiphenyl ether) and methyl mercury enhances developmental neurotoxic defects. Toxicol. Sci. 101:275–285.

Fonnum, F., E. Mariussen, and T. Reistad. 2006. Molecular mechanisms involved in the toxic effects of polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs). J. Toxicol. Environ. Health A 69:21–35.

Ford, J. K. B., and G. M. Ellis. 2006. Selective foraging by fish-eating killer whales *Orcinus orca* in British Columbia. Mar. Ecol. Prog. Ser. 316:185–199.

Ford, J. K. B., G. M. Ellis, L. G. Barrett-Lennard, A. B. Morton, R. S. Palm, K. C. Balcomb III. 1998. Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. Can. J. Zool. 76:1456–1471.

Ford, J. K. B., G. M. Ellis, and P. F. Olesiuk. 2005. Linking prey and population dynamics: Did food limitation cause recent declines of 'resident' killer whales (*Orcinus orca*) in British Columbia? Fisheries and Oceans Canada Report no. 2005/042, 31 p.

Ford, J. K., G. M. Ellis, P. F. Olesiuk, and K. C. Balcomb. 2010. Linking killer whale survival and prey abundance: Food limitation in the oceans' apex predator? Biol. Lett. 6:139–142.

Ford, M. J., J. Hempelmann, M. B. Hanson, K. L. Ayres, R. W. Baird, C. K. Emmons, J. I. Lundin, G. S. Schorr, S. K. Wasser, and L. K. Park. 2016. Estimation of a killer whale (*Orcinus orca*) population's diet using sequencing analysis of DNA from feces. PLoS ONE. 11(1): e01444956. doi:10.1371/journal.pone.0144956

Fossi, M. C., S. Casini, and L. Marsili. 1999. Nondestructive biomarkers of exposure to endocrine disrupting chemicals in endangered species of wildlife. Chemosphere 39:1273–1285.

Fossi, M. C., J. Urban, S. Casini, S. Maltese, G. Spinsanti, C. Panti, S. Porcelloni, S. Panigada, G. Lauriano, C. Niño-Torrez, L. Rojas-Bracho, B. Jimenez, J. Munoz-Arnanz, and L. Marsili. 2010. A multi-trial diagnostic tool in fin whale (*Balaenoptera physalus*) skin biopsies of the Pelagos Sanctuary (Mediterranean Sea) and the Gulf of California (Mexico). Mar. Environ. Res. 69 Suppl:S17–20.

Fowler, P. A., M. Bellingham, K. D. Sinclair, N. P. Evans, P. Pocar, B. Fischer, K. Schaedlich, J. S. Schmidt, M. R. Amezaga, S. Bhattacharya, S. M. Rhind, and P. J. O'Shaughnessy. 2012. Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. Mol. Cell. Endocrinol. 355:231–239.

Fresh, K. L., R. D. Cardwell, and R. R. Koons. 1981. Food habits of Pacific salmon, baitfish, and their potential competitors and predators in the marine waters of Washington, August 1978 to September 1979. Olympia, Washington: Washington State Department of Fisheries. Report no. 145.

Frost, K. J., C. A. Manen, and T. L. Wade. 1994. Petroleum hydrocarbons in tissues of harbor seals from Prince William Sound and the Gulf of Alaska. *In* T. R. Loughlin (ed.), Marine mammals and the *Exxon Valdez*, pp. 331–358. Academic Press, San Diego, CA.

Frouin, H., M. Lebeuf, M. Hammill, S. Masson, and M. Fournier. 2010. Effects of individual polybrominated diphenyl ether (PBDE) congeners on harbour seal immune cells in vitro. Mar. Pollut. Bull. 60:291–298.

Fry, D. M. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Perspect. 103(Supplement 7):165–171.

Fukushima, M. and Kawai, S., 1981. Variation of organochlorine residue concentration and burden in striped dolphin (*Stenella coeruleoalba*) with growth. *In* T. Fujiyama (ed.), Studies on the levels of organochlorine compounds and heavy metals in marine organisms, pp. 97–114. University of Ryukyus, Japan.

Gao, P., P. He, A. Wang, T. Xia, Z. Xu, Q. Niu, L. Guo, and X. Chen. 2009. Effects of PCB-153 on oxidative stress and 8-OHdG content induced by PBDE-47 in human neuroblastoma cells in vitro. Wei Sheng Yan Jiu 38: 513–515.

Gaydos, J. K., K. C. Balcomb, R. W. Osborne, and L. Dierauf. 2004. Evaluating potential infectious disease threats for southern resident killer whales, *Orcinus orca*: A model for endangered species. Biol. Conserv. 117:253–262.

Geraci, J. R., and D. J. St. Aubin. 1990. Sea mammals and oil: Confronting the risks. Academic Press, San Diego, CA.

Gilmartin, W. G., R. L. Delong, A. W. Smith, J. C. Sweeney, B. W. De Lappe, R. W. Risebrough, L. A. Griner, M. D. Dailey, and D. B. Peakall. 1976. Premature parturition in the California sea lion. J. Wildl. Dis. 12:104–115.

Gockel, C. K., and T. M. Mongillo. 2013. Potential effects of PBDEs on Puget Sound and Southern Resident killer whales: A report on the technical workgroups and policy forum. EPA and NOAA, 20 p.

Goldstein, T., T. S. Zabka, R. DeLong, E. A. Wheeler, G. Ylitalo, S. Bargu, M. Silver, T. Leighfield, F. Van Dolah, G. Langlois, I. Sidor, J. L. Dunn, and F. M. D. Gulland. 2009. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. J. Wildl. Dis. 45(1):91–108.

Grant, S. C. H., and P. S. Ross. 2002. Southern Resident killer whales at risk: Toxic chemicals in the British Columbia and Washington environment. Can. Tech. Rep. Fish. Aquat. Sci. 2412:1–111.

Gregory, M., and D. G. Cyr. 2003. Effects of environmental contaminants on the endocrine system of marine mammals. *In* J. G. Vos, G. D. Bossart, M. Fournier, and T. O'Shea (eds.), Toxicology of marine mammals, pp. 67–81. Taylor and Francis, New York.

Greig, D. J., G. M. Ylitalo, A. J. Hall, D. A. Fauquier, and F. Gulland. 2007. Transplacental transfer of organochlorines in California sea lions (*Zalophus californianus*). Environ. Toxicol. Chem. 26:37–44.

Hale, R. C., M. Alaee, J. B. Manchester-Neesvig, H. M. Stapleton, and M. G. Ikonomou. 2003. Polybrominated diphenyl ether flame retardants in the North American environment. Environ. Int. 29:771–779.

Hall, A. J., K. Hugunin, R. Deaville, R. J. Law, C. R. Allchin, and P. D. Jepson. 2006a. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (*Phocoena phocoena*): A case–control approach. Environ. Health Perspect. 114:704–711.

Hall, A. J., O. I. Kalantzi, and G. O. Thomas. 2003. Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life—are they thyroid hormone endocrine disrupters? Environ. Pollut. 126:29–37.

Hall, A. J., B. J. McConnell, T. K. Rowles, A. Aguilar, A. Borrell, L. Schwacke, P. J. Reijnders, and R. S. Wells. 2006b. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environ. Health Perspect. 114 (Suppl. 1):60–64.

Hallgren, S., and P. O. Darnerud. 2002. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats—testing interactions and mechanisms for thyroid hormone effects. Toxicology 177:227–243.

Hallgren, S., T. Sinjari, H. Hakansson, and P. O. Darnerud. 2001. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch. Toxicol. 75:200–208.

Hammond, J. A., A. J. Hall, and E. A. Dyrynda. 2005. Comparison of polychlorinated biphenyl (PCB) induced effects on innate immune functions in harbour and grey seals. Aquat. Toxicol. 74:126–138.

Hanson, M. B., R. W. Baird, J. K. B. Ford, J. Hempelmann-Halos, D. M. Van Doornik, J. R. Candy, C. K. Emmons, G. S. Schorr, B. Gisborne, K. L. Ayres, S. K. Wasser, K. C. Balcomb, K. Balcomb-Bartok, J. G. Sneva, and M. J. Ford. 2010. Species and stock identification of prey consumed by endangered southern resident killer whales in their summer range. Endanger. Spec. Res. 11:69–82.

Hanson, M. B., C. K. Emmons, E. J. Ward, J. A. Nystuen, and M. O. Lammers. 2013. Assessing the coastal occurrence of endangered killer whales using autonomous passive acoustic recorders. J. Acoust. Soc. Am. 134:3486–3495.

Hany, J., H. Lilienthal, A. Sarasin, A. Roth-Harer, A. Fastabend, L. Dunemann, W. Lichtensteiger,and G. Winneke. 1999. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: Effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol. Appl. Pharmacol. 158:231–243.

Haraguchi, K., Y. Hisamichi, and T. Endo. 2009. Accumulation and mother-to-calf transfer of anthropogenic and natural organohalogens in killer whales (*Orcinus orca*) stranded on the Pacific coast of Japan. Sci. Total Environ. 407:2853–2859.

Hardell, S., H. Tilander, G. Welfinger-Smith, J. Burger, and D. O. Carpenter. 2010. Levels of polychlorinated biphenyls (PCBs) and three organochlorine pesticides in fish from the Aleutian Islands of Alaska. PLoS ONE 5:e12396.

Hart Crowser, Inc., Ecology, EPA, and Puget Sound Partnership. 2007. Phase I: Initial estimate of toxic chemical loadings to Puget Sound. Ecology Publ. No. 07-10-079.

Hauser, D. D. W., M. G. Logsdon, E. E. Holmes, G. R. VanBlaricom, and R. W. Osborne. 2007. Summer distribution patterns of southern resident killer whales *Orcinus orca*: Core areas and spatial segregation of social groups. Mar. Ecol. Prog. Ser. 351:301–310.

Hayward, D., J. Wong, and A. J. Krynitsky. 2007. Polybrominated diphenyl ethers and polychlorinated biphenyls in commercially wild caught and farm-raised fish fillets in the United States. Environ. Res. 103:46–54.

He, P., A. G. Wang, T. Xia, P. Gao, Q. Niu, L. J. Guo, and X. M. Chen. 2009. Mechanisms underlying the developmental neurotoxic effect of PBDE-47 and the enhanced toxicity associated with its combination with PCB153 in rats. Neurotoxicology 30:1088–1095.

He W., A. Wang, T. Xia, P. Gao, B. Xu, Z. Xu, P. He, and X. Chen. 2010. Cytogenotoxicity induced by PBDE-47 combined with PCB153 treatment in SH-SY5Y cells. Environ. Toxicol. 25:564–572.

Healey, M. C. 1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type Chinook salmon, *Oncorhynchus tshawytscha*. Can. Field Nat. 97:427–433.

Heimlich-Boran, J. R. 1988. Behavioral ecology of killer whales (*Orcinus orca)* in the Pacific Northwest. Can. J. Zool. 66:565–578.

Heintz, R. A., S. D. Rice, A. C. Werthheimer, R. F. Bradshaw, F. P. Thrower, J. E. Joyce, and J. W. Short. 2000. Delayed effects on growth and marine survival of pink salmon *Onchorynchus gorbuscha* after exposure to crude oil during embryonic development. Mar. Ecol. Prog. Ser. 208:205–216.

Henderson, M. A., and C. C. Graham. 1998. History and current status of Pacific salmon in British Columbia. *In* North Pacific Anadromous Fish Commission, Bulletin no. 1: Proceedings of the 1996 symposium on assessment and status of Pacific Rim salmonid stocks, pp. 13–22. Sapporo, Japan.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Can. J. Zool. 77:1663–1675.

Hickie, B. E., P. S. Ross, R. W. Macdonald, and J. K. Ford. 2007. Killer whales (*Orcinus orca*) face protracted health risks associated with lifetime exposure to PCBs. Environ. Sci. Technol. 41:6613–6619.

Higgs, D. A., J. S. MacDonald, C. D. Levings, and B. S. Dosanjih. 1995. Nutrition and feeding habits in relation to life history stage. *In* C. Groot, L. Margolis, and W. C. Clarke (eds.), Physiological ecology of Pacific salmon, pp. 157–315. University of British Columbia Press, Vancouver, BC, Canada..

Hilborn, R., S. P. Cox, F. M. D. Gulland, D. G. Hankin, N. T. Hobbs, D. E. Schindler, and A. W. Trites. 2012. The effects of salmon fisheries on Southern Resident killer whales: Final Report of the independent science panel. Prepared with the assistance of D. R. Marmorek and A. W. Hall, ESSA Technologies Ltd., Vancouver, BC, Canada, for National Marine Fisheries Service and Fisheries and Oceans Canada.

Hites, R. A. 2004. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. Environ. Sci. Technol. 38:945–956.

Hites, R. A., J. A. Foran, D. O. Carpenter, M. C. Hamilton, B. A. Knuth, and S. J. Schwager. 2004a. Global assessment of organic contaminants in farmed salmon. Science 303:226–229.

Hites, R. A., J. A. Foran, S. J. Schwager, B. A. Knuth, M. C. Hamilton, and D. O. Carpenter. 2004b. Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. Environ. Sci. Technol. 38:4945–4949.

Hoekstra, P. F., R. J. Letcher, T. M. O'Hara, S. M. Backus, K. R. Solomon, and D. C. G. Muir. 2003. Hydroxylated and methylsulfone-containing metabolites of polychlorinated biphenyls in the plasma and blubber of bowhead whales (*Balaena mysticetus*). Environ. Toxicol. Chem. 22:2650–2658.

Holsbeek, L., C. R. Joiris, V. Debacker, I. B. Ali, P. Roose, J. Nellissen, S. Gobert, J. Bouquegneau, and M. Bossicart. 1999. Heavy metals, organochlorines and polycyclic aromatic hydrocarbons in sperm whales stranded in the southern North Sea during the 1994/1995 winter. Mar. Pollut. Bull. 38(4):304–313.

Hooper, K., and T. A. McDonald. 2000. The PBDEs: An emerging environmental challenge and another reason for breast-milk monitoring programs. Environ. Health Perspect. 108:387–392.

Houde, M., P. F. Hoekstra, K. R. Solomon, and D. C. Muir. 2005. Organohalogen contaminants in delphinoid cetaceans. Rev. Environ. Contam. Toxicol. 184:1–57.

Hrgovcic, M. J., C. F. Tessmer, D. M. Mumford, P. S. Ong, J. F. Gamble, and C. C. Shullenberger. 1975. Interpreting serum copper levels in Hodgkin's disease. Tex. Med. J. 71:53–63.

Ikonomou, M. G., S. Rayne, and N. F. Crewe. 2007. Concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), and polybrominated diphenyl ethers (PBDEs) as functions of sample depth in killer whale (*Orcinus orca*) blubber. J. Appl. Sci. Environ. Manag. 11:147–151.

Ikonomou, M. G., S. Rayne, M. Fischer, M. P. Fernandez, and W. Cretney. 2002. Occurrence and congener profiles of polybrominated diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. Chemosphere 46:649–663.

Incardona, J. P., T. K. Collier, and N. L. Scholz. 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196:191–205.

Incardona, J. P., C. A. Vines, B. F. Anulacion, D. H. Baldwin, H. L. Day, B. L. French, J. S. Labenia, T. L. Linbo, M. S. Myers, O. P. Olson, C. A. Sloan, S. Sol, F. J. Griffin, K. Menard, S. G. Morgan, J. E. West, T. K. Collier, G. M. Ylitalo, G. N. Cherr, and N. L. Scholz. 2012. Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 *Cosco Busan* oil spill in San Francisco Bay. Proc. Natl. Acad. Sci. U.S.A. 109(2):E51–58.

Inoue, Y., T. Itou, T. Jimbo, Y. Syouji, K. Ueda, and T. Sakai. 2001. Molecular cloning and functional expression of bottle-nosed dolphin (*Tursiops truncatus*) interleukin-1 receptor antagonist. Vet. Immunol. Immunopathol. 78:131–141.

Isobe, T., T. Oshihoi, H. Hamada, K. Nakayama, T. K. Yamada, Y. Tajima, M. Amano, and S. Tanabe. 2011. Contamination status of POPs and BFRs and relationship with parasitic infection in finless porpoises (*Neophocaena phocaenoides*) from Seto Inland Sea and Omura Bay, Japan. Mar. Pollut. Bull. 63:564–571.

Iwata, H., S. Tanabe, N. Sakai, and R. Tatsukawa. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ. Sci. Technol. 27:1080–1098.

Jaber, J. R., A. Fernandez, P. Herraez, A. Espinosa de los Monteros, G. A. Ramirez, P. M. Garcia, T. Fernandez, M. Arbelo, and J. Perez. 2003a. Cross-reactivity of human and bovine antibodies in striped dolphin paraffin wax-embedded tissues. Vet. Immunol. Immunopathol. 96:65–72.

Jaber, J. R., J. Perez, M. Arbelo, P. Herraez, A. Espinosa de los Monteros, F. Rodriguez, T. Fernandez, and A. Fernandez. 2003b. Immunophenotypic characterization of hepatic inflammatory cell infiltrates in common dolphins (*Delphinus delphis*). J. Comp. Pathol. 129:226–230.

Jarman, W. M., R. J. Norstrom, D. C. G. Muir, B. Rosenberg, M. Simon, and R. W. Baird. 1996. Levels of organochlorine compounds, including PCDDs and PCDFs, in the blubber of cetaceans from the west coast of North America. Mar. Pollut. Bull. 32:426–436.

Jarvis, E., K. Schiff, L. Sabin, and M. J. Allen. 2007. Chlorinated hydrocarbons in pelagic forage fishes and squid of the Southern California Bight. Environ. Toxicol. Chem. 26(11):2290–2298.

Jenssen, B. M., O. Haugen, E. G. Sørmo, and J. U. Skaare. 2003. Negative relationship between PCBs and plasma retinol in low-contaminated free-ranging gray seal pups (*Halichoerus grypus).* Environ. Res. 93:79–87.

Jepson, P. D., P. M. Bennett, R. Deaville, C. R. Allchin, J. R. Baker, and R. J. Law. 2005. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ. Toxicol. Chem. 24:238–248.

Jepson, P. D., R. Deaville, J. L. Barber, À. Aguilar, A. Borrell, S. Murphy, J. Barry, A. Brownlow, J. Barnett, S. Berrow, A. A. Cunningham, N. J. Davison, M. Doeschate, R. Esteban, M. Ferreira, A. D. Foote, T. Genov, J. Giménez, J. Loveridge, Á. Llavona, V. Martin, D. L. Maxwell, A. Papachlimitzou, R. Penrose, M. W. Perkins, B. Smith, R. Stephanis, N. Tregenza, P. Verborgh, A. Fernandez, and R. J. Law. 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci. Rep. 6:18573. doi:10.1038/srep18573

Johannessen, S. C., R. W. Macdonald, C. A. Wright, B. Burd, D. P. Shaw, and A. van Roodselaar. 2008. Joined by geochemistry, divided by history: PCBs and PBDEs in Strait of Georgia sediments. Mar. Environ. Res. 66 (Suppl):S112–120.

Johansson, N., H. Viberg, A. Fredriksson, and P. Eriksson. 2008. Neonatal exposure to deca-brominated diphenyl ether (PBDE 209) causes dose–response changes in spontaneous behaviour and cholinergic susceptibility in adult mice. Neurotoxicology 29:911–919.

Johnson, L. L., G. M. Ylitalo, M. R. Arkoosh, A. N. Kagley, C. L. Stafford, J. L. Bolton, J Buzitis, B. Anulacion, and T. K. Collier. 2007. Contaminant exposure in outmigrant juvenile salmon from Pacific Northwest estuaries. Environ. Monit. Assess. 124:167–194.

Johnson, S. P., and D. E. Schindler. 2008. Trophic ecology of Pacific salmon (*Oncorhynchus* spp.) in the ocean: A synthesis of stable isotope research. Ecol. Res. 24:885–863.

Kajiwara, N., S. Kamikawa, M. Amano, A. Hayano, T. K. Yamada, Miyazaki, N., and Tanabe, S. 2008. Polybrominated diphenyl ethers (PBDES) and organochlorines in melon-headed whales, *Peponocephala electra*, mass stranded along the Japanese coasts: Maternal transfer and temporal trend. Environ. Pollut. 156:106–114.

Kajiwara, N., T. Kunisue, S. Kamikawa, Y. Ochi, S. Yano, and S. Tanabe. 2006. Organohalogen and organotin compounds in killer whales mass-stranded in the Shiretoko Peninsula, Hokkaido, Japan. Mar. Pollut. Bull. 52:1066–1076.

Kannan, K., A. L. Blankenship, P. D. Jones, and J. P. Giesy. 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk Assess. 6:181–201.

Kannan, K., J. Koistinen, K. Beckmen, T. Evans, J. F. Gorzelany, K. J. Hansen, P. D. Jones, E. Helle, M. Nyman, and J. P. Giesy. 2001. Accumulation of perfluorooctane sulfonate in marine mammals. Environ. Sci. Technol. 35:1593–1598.

Kannan, K., E. Perrotta, N. J. Thomas, and K. M. Aldous. 2007. A comparative analysis of polybrominated diphenyl ethers and polychlorinated biphenyls in southern sea otters that died of infectious diseases and noninfectious causes. Arch. Environ. Contam. Toxicol. 53:293–302.

Kawashima, M., M. Kuwamura, M. Takeya, and J. Yamate. 2004a. Morphologic characteristics of pulmonary macrophages in cetaceans: Particular reference to pulmonary intravascular macrophages as a newly identified type. Vet. Pathol. 41:682–686.

Kawashima, M., M. Nakanishi, M. Kuwamura, M. Takeya, and J. Yamate. 2004b. Distributive and phagocytic characteristics of hepatic macrophages in five cetaceans belonging to Delphinidae and Ziphiidae. J. Vet. Med. Sci. 66:671–680.

Kawashima, M., M. Nakanishi, M. Kuwamura, M. Takeya, and J. Yamate. 2004c. Immunohistochemical detection of macrophages in the short-finned pilot whale (*Globicephala macrorhynchus*) and Risso's dolphin (*Grampus griseus*). J. Comp. Pathol. 130:32–40.

Kellar, N. M., M. L. Trego, S. J. Chivers, and F. I. Archer. 2013. Pregnancy patterns of pantropical spotted dolphins (*Stenella attenuata*) in eastern tropical Pacific determined from hormonal analysis of blubber biopsies and correlations with the purse-seine tuna fishery. Mar. Biol. 160:3113–3124.

Kelly, B. C., S. L. Gray, M. G. Ikonomou, J. S. MacDonald, S. M. Bandiera, and E. G. Hrycay. 2007. Lipid reserve dynamics and magnification of persistent organic pollutants in spawning sockeye salmon (*Oncorhynchus nerka*) from the Fraser River, British Columbia. Environ. Sci. Technol. 41:3083–3089.

Kiciński, M., M. K. Viaene, E. Den Hond, G. Schoeters, A. Covaci, A. C. Dirtu, V. Nelen, L. Bruckers, K. Croes, I. Sioen, W. Baeyens, N. Van Larebeke, and T. S. Nawrot. 2012. Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study. Environ. Health 11:86.

Kimbrough, K. L., W. E. Johnson, G. G. Lauenstein, J. D. Christensen, and D.A. Apeti. 2008. An assessment of two decades of contaminant monitoring in the nation's coastal zone. U.S. Dept. Commer., NOAA Tech. Memo. NOS-NCCOS-74, 105 pp.

Kinross, J. M., A. W. Darzi, and J. K. Nicholson. 2011. Gut microbiome–host interactions in health and disease. Genome Med. 3(14):1–12.

Klaassen, C. D., J. Liu, and B. A. Diwan. 2009. Metallothionein protection of cadmium toxicity. Toxicol. Appl. Pharmacol. 238:215–220.

Kodavanti, P. R., C. G. Coburn, V. C. Moser, R. C. MacPhail, S. E. Fenton, T. E. Stoker, J. L. Rayner, K. Kannan, and L. S. Birnbaum. 2010. Developmental exposure to a commercial PBDE mixture, DE-71: Neurobehavioral, hormonal, and reproductive effects. Toxicol. Sci. 116:297–312.

Komohara, Y., J. Hirahara, T. Horikawa, K. Kawamura, E. Kiyota, N. Sakashita, N. Araki, and M. Takeya. 2006. AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an anti-inflammatory macrophage phenotype. J. Histochem. Cytochem. 54:763–771.

Krahn, M. M., M. B. Hanson, R. W. Baird, R. H. Boyer, D. G. Burrows, C. K. Emmons, J. K. B. Ford, L. L. Jones, D. P. Noren, P. S. Ross, G. S. Schorr, and T. K. Collier. 2007a. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. Mar. Pollut. Bull. 54:1903–1911.

Krahn, M. M., M. B. Hanson, G. S. Schorr, C. K. Emmons, D. G. Burrows, J. L. Bolton, R. W. Baird, and G. M. Ylitalo. 2009. Effects of age, sex and reproductive status on persistent organic pollutant concentrations in "Southern Resident" killer whales. Mar. Pollut. Bull. 58:1522–1529.

Krahn, M. M., D. P. Herman, C. Matkin, J. W. Durban, L. Barrett-Lennard, D. G. Burrows, M. Dahlheim, N. Black, R. G. LeDuc, and P. R. Wade. 2007b. Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Mar. Environ. Res. 63:91–114.

Krahn, M. M., D. P. Herman, G. M. Ylitalo, C. A. Sloan, D. G. Burrows, R. C. Hobbs, B. A. Mahoney, G. K. Yanagida, J. Calambokidis, and S. E. Moore. 2004. Stratification of lipids, fatty acids and organochlorine contaminants in blubber of white whales and killer whales. J. Cetacean Res. Manag. 6:175–189.

Krahn, M. M., M. S. Myers, D. G. Burrows, and D. C. Malins. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14:633–646.

Krahn, M. M, R. Pitman, D. G. Burrows, D. P. Herman, and R. W. Pearce. 2008. Use of chemical tracers to assess diet and persistent organic pollutants in Antarctic Type C killer whales. Mar. Mamm. Sci. 24:643–663.

Krahn, M. M., and J. E. Stein. 1998. Assessing exposure of marine biota and habitats to petroleum compounds. Anal. Chem. 70:186a–192a.

Krahn, M. M., P. R. Wade, S. T. Kalinowski, M. E. Dahlheim, B. L. Taylor, M. B. Hanson, G. M. Ylitalo, R. P. Angliss, J. E. Stein, and R. S. Waples. 2002. Status review of Southern Resident killer whales (*Orcinus orca*) under the Endangered Species Act. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-54, 133 p.

Krahn, M. M., G. M. Ylitalo, D. G. Burrows, J. Calambokidis, S. E. Moore, M. Gosho, P. Gearin, P. D. Plesha, R. L. Brownell, S. A. Blokhin, K. L. Tilbury, T. Rowles, and J. E. Stein. 2001. Organochlorine contaminant concentrations and lipid profiles in eastern North Pacific gray whales (*Eschrichtius robustus*). J. Cetacean Res. Manag. 3:19–29.

Krahn, M. M., G. M. Ylitalo, J. Buzitis, S. Chan, and U. Varanasi. 1993. Rapid high-performance liquid chromatographic methods that screen for aromatic compounds in environmental samples. J. Chromatogr. 642:15–32.

Kumar, D., and D. F. Cowan. 1994. Cross-reactivity of antibodies to human antigens with tissues of the bottlenose dolphin, *Tursiops truncatus*, using immunoperoxidase techniques. Mar. Mamm. Sci. 10:188–194.

Kuriyama, S. N., C. E. Talsness, K. Grote, and I. Chahoud. 2005. Developmental exposure to low dose PBDE 99: Effects on male fertility and neurobehavior in rat offspring. Environ. Health Perspect. 113:149–154.

Law, R. J., J. Barry, P. Bersuder, J. L. Barber, R. Deaville, R. J. Reid, and P. D. Jepson. 2010. Levels and trends of brominated diphenyl ethers in blubber of harbor porpoises (*Phocoena phocoena*) from the U.K., 1992–2008. Environ. Sci. Technol. 44:4447–4451.

Lebeuf, M., B. Gouteux, L. Measures, and S. Trottier. 2004. Levels and temporal trends (1988–1999) of polybrominated diphenyl ethers in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada. Environ. Sci. Technol. 38:2971–2977.

Lee, J. S., S. Tanabe, H. Umino, R. Tatsukawa, T. R. Loughlin, and D. C. Calkins. 1996. Persistent organochlorines in Steller sea lion (*Eumetopias jubatus*) from the bulk of Alaska and the Bering Sea, 1976–1981. Mar. Pollut. Bull. 32:535–544.

Lefkovitz, L. F., V. I. Cullinan, and E. A. Crecelius. 1997. Historical trends in the accumulation of chemicals in Puget Sound. U.S. Dept. Commer., NOAA Tech. Memo. NOS-ORCA-111, 60 p.

Legler, J. 2008. New insights into the endocrine disrupting effects of brominated flame retardants. Chemosphere 73:216–222.

Legler, J., and A. Brouwer. 2003. Are brominated flame retardants endocrine disruptors? Environ. Int. 29:879–885.

Letcher, R. J., J. O. Bustnes, R. Dietz, B. M. Jenssen, E. H. Jorgensen, C. Sonne, J. Verreault, M. M. Vijayan, and G. W. Gabrielsen. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408:2995–3043.

Leung, C. C. M., T. A. Jefferson, S. K. Hung, G. J. Zheng, L. W. Y. Yeung, B. J. Richardson, and P. K. S. Lam. 2005. Petroleum hydrocarbons, polycyclic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls in tissues of Indo-Pacific humpback dolphins from south China waters. Mar. Pollut. Bull. 50:1713–1744.

Levin, M., S. De Guise, and P.S. Ross. 2005. Association between lymphocyte proliferation and polychlorinated biphenyls in free-ranging harbor seal (*Phoca vitulina*) pups from British Columbia, Canada. Environ. Toxicol. Chem. 24:1247–1252.

Levin, M., H. Leibrecht, C. Mori, D. Jessup, S. de Guise. 2007. Immunomodulatory effects of organochlorine mixtures upon in vitro exposure of peripheral blood leukocytes differ between free-ranging and captive southern sea otters (*Enhydra lutris*). Vet. Immunol. Immunopathol. 119:269–277.

Lieberg-Clark, P., C. E. Bacon, S. A. Burns, W. M. Jarman, and B. J. Le Boeuf. 1995. DDT in California sea-lions: A follow-up study after twenty years. Mar. Pollut. Bull. 30:744–745.

Lindstrom, G., H. Wingfors, M. Dam, and B. van Bavel. 1999. Identification of 19 polybrominated diphenyl ethers (PBDEs) in long-finned pilot whale (*Globicephala melas*) from the Atlantic. Arch. Environ. Contam. Toxicol. 36:355–363.

Louis, C., A. C. Dirtu, M. Stas, Y. Guiot, G. Malarvannan, K. Das, D. P. Costa, D. E. Crocker, A. Covaci, and C. Debier. 2014. Mobilisation of lipophilic pollutants from blubber in northern elephant seal pups (*Mirounga angustirostris*) during the post-weaning fast. Environ. Res. 132:438–448.

Lundin, J. I., R. L. Dills, G. M. Ylitalo, M. B. Hanson, C. K. Emmons, G. S. Schoor, J. Ahmad, J. A. Hempelmann, K. M. Parsons, and S. K. Wasser. 2015. Persistent organic pollutant determination in killer whale scat samples: Optimization of a gas chromatography/mass spectrometry method and application to field samples. Arch. Environ. Contam. Toxicol. 70(1):9–19. doi:10.1007/s00244-015-0218-8

Lyche, J. L., H. J. S. Larsen, J. U. Skaare, A. Tverdal, G. M. Johansen, and E. Ropstad. 2006. Perinatal exposure to low doses of PCB-153 and PCB-126 affects maternal and neonatal immunity in goat kids. J. Toxicol. Environ. Health, Part A: Current Issues. 69:139–158.

Mackenzie, C. A., A. Lockridge, and M. Keith. 2005. Declining sex ratio in a First Nation community. Environ. Health Perspect. 113:1295–1298.

Marsili, L., A. Caruso A, M. C. Fossi, M. Zanardelli, E. Politi, and S. Focardi. 2001. Polycyclic aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans. Chemosphere 44:147–154.

Martineau, D., S. de Guise, M. Fournier, L. Shugart, C. Girard, A. Lagacé, and P. Béland. 1994. Pathology and toxicology of beluga whales from the St. Lawrence estuary, Quebec, Canada. Past, present and future. Sci. Total Environ. 154:201–215.

Matkin, C. O., J. W. Testa, G. M. Ellis, and E. L. Saulitis. 2014. Life history and population dynamics of southern Alaska resident killer whales (*Orcinus orca*). Mar. Mamm. Sci. 30:460–479.

McCluskey, S. 2006. Space use patterns and population trends of Southern Resident killer whales (*Orcinus orca*) in relation to distribution and abundance of Pacific salmon (*Oncorhynchus* spp.) in the inland marine waters of Washington state and British Columbia. Thesis. University of Washington, Seattle, WA.

McHugh, B., R. J. Law, C. R. Allchin, E. Rogan, S. Murphy, M. B. Foley, D. Glynn, and E. McGovern. 2007. Bioaccumulation and enantiomeric profiling of organochlorine pesticides and persistent organic pollutants in the killer whale (*Orcinus orca*) from British and Irish waters. Mar. Pollut. Bull. 54:1724–1731.

Meador, J. P., D. Ernest, A. A. Hohn, K. Tilbury, J. Gorzelany, G. Worthy, and J. E. Stein. 1999. Comparison of elements in bottlenose dolphins stranded on the beaches of Texas and Florida in the gulf of Mexico over a one-year period. Arch. Environ. Contam. Toxicol. 36:87–98.

Meador, J. P., F. C. Sommers, G. M. Ylitalo, and C. A. Sloan. 2006. Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tschawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Can. J. Fish. Aquat. Sci. 63:2364–2376.

Meador, J. P., J. E. Stein, W. L. Reichert, and U. Varanasi. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143:79–165.

Mearns, A. J. 1988. Inventory band trends of chlorinated pesticide and PCB concentrations in United States fishes and invertebrates. Aquat. Toxicol. 11:418–418.

Meerts, I. A., R. J. Letcher, S. Hoving, G. Marsh, A. Bergman, J. G. Lemmen, B. van der Burg, and A. Brouwer. 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. Environ. Health Perspect. 109:399–407.

Meerts, I. A., J. J. van Zanden, E. A. Luijks, I. van Leeuwen-Bol, G. Marsh, E. Jakobsson, A. Bergman, and A. Brouwer. 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol. Sci. 56:95–104.

Miller, V. M., S. Sanchez-Morrissey, K. O. Brosch, and R. F. Seegal. 2012. Developmental coexposure to polychlorinated biphenyls and polybrominated diphenyl ethers has additive effects on circulating thyroxine levels in rats. Toxicol. Sci. 127:76–83.

Missildine, B. R., R. J. Peters, G. Chin-Leo, and D. Houck. 2005. Polychlorinated biphenyl concentrations in adult chinook salmon (*Oncorhynchus tshawytscha*) returning to coastal and Puget Sound hatcheries of Washington State. Environ. Sci. Technol. 39:6944–6951.

Mongillo, T. M., E. E. Holmes, D. P. Noren, G. R. VanBlaricom, A. E. Punt, S. M. O'Neill, G. M. Ylitalo, M. B. Hanson, and P. S. Ross. 2012. Predicted polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) accumulation in southern resident killer whales. Mar. Ecol. Prog. Ser. 453:263–277.

Montory, M., E. Habit, P. Fernandez, J. O. Grimalt, and R. Barra. 2010. PCBs and PBDEs in wild Chinook salmon (*Oncorhynchus tshawytscha*) in the Northern Patagonia, Chile. Chemosphere 78:1193–1199.

Moon, H., Y. An, K. Park, S. Choi, D. Moon, M. Choi, and H. Choi. 2011. Occurrence and accumulation features of polycyclic aromatic hydrocarbons and synthetic musk compounds in finless porpoise (*Neophocaena phocaenoides*) from Korean coastal waters. Mar. Pollut. Bull. 62:1963–1968.

Mori, C., B. Morsey, M. Levin, T. S. Gorton, and S. de Guise. 2008. Effects of organochlorines, individually and in mixtures, on B-cell proliferation in marine mammals and mice. J. Toxicol. Environ. Health, Part A: Current Issues. 71:266–275.

Mos, L., M. Tabuchi, N. Dangerfield, S. J. Jeffries, B. F. Koop, and P. S. Ross. 2007. Contaminant-associated disruption of vitamin A and its receptor (retinoic acid receptor alpha) in free-ranging harbour seals (*Phoca vitulina*). Aquat. Toxicol. 81:319–328.

Moura, A. E., J. G. Kenny, R. Chaudhuri, M. A. Hughes, A. J. Welch, R. R. Reisinger, P. J. Nico de Bruyn, M. E. Dahlheim, N. Hall, and A. R. Hoelzel. 2014. Population genomics of the killer whale indicates ecotype evolution in sympatry involving both selection and drift. Mol. Ecol. 23:5179–5192.

Murphy, S., G. J. Pierce, R. J. Law, P. Bersuder, P. D. Jepson, J. A. Learmonth, M. Addink, W. Dabin, M. B. Santos, R. Deaville, B. N. Zegers, A. Mets, E. Rogan, V. Ridoux, R. J. Reid, C. Smeenk, T. Jauniaux, A. Løpez, J. M. Alonso Farré, A. F. Gonzá-Hartmann, C. Lockyer, and J. P. Boon. 2010. Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises. J. Northwest Atl. Fish. Sci. 42:153–173.

Myers, M. J., L. D. Rea,and S. Atkinson. 2006. The effects of age, season and geographic region on thyroid hormones in Steller sea lions (*Eumetopias jubatus*). Comp. Biochem. Physiol. Part A 145:90–98.

Neale, J. C. C., F. M. D. Gulland, K. R. Schmelzer, J. T. Harvey, E. A. Berg, S. G. Allen, D. J. Greig, E. K. Grigg, and R. S. Tjeerdema. 2005. Contaminant loads and hematological correlates in the harbor seal (*Phoca vitulina*) of San Francisco Bay, California. J. Toxicol. Environ. Health, Part A: Current Issues 68:617–633.

Nielsen, J. B., F. Nielsen, P. J. Jorgensen, and P. Grandjean. 2000. Toxic metals and selenium in blood from pilot whales (*Globicephala melas*) and sperm whales (*Physeter catodon*). Mar. Pollut. Bull. 40:348–351.

Niño-Torres, C. A., T. Zenteno-Savin, S. C. Gardner, and R. J. Urban. 2010. Organochlorine pesticides and polychlorinated biphenyls in fin whales (*Balaenoptera physalus*) from the Gulf of California. Environ. Toxicol. 25:381–390.

NMFS (National Marine Fisheries Services). 2005. Endangered and threatened wildlife and plants: Endangered status for Southern Resident killer whales. Federal Register (Docket No. 041213348-5285-02, 18 November 2005) 70(217):69903–69912.

NMFS (National Marine Fisheries Services). 2006. Endangered and threatened species; designation of critical habitat for Southern Resident killer whales. Federal Register (Docket No. 060228057-6283-02, 29 November 2006) 71(229):69054–69070.

NMFS (National Marine Fisheries Service). 2008. Final recovery plan for Southern Resident killer whales (*Orcinus orca*). Federal Register (73 FR 4176, 24 January 2008).

Norén, K., and D. Meironyté. 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. Chemosphere 40:1111–1123.

Noren, D. P., and J. A. Mocklin. 2012. Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Mar. Mamm. Sci. 28:154–199.

Oenga, G. N., D. C. Spink, and D. O. Carpenter. 2004. TCDD and PCBs inhibit breast cancer cell proliferation in vitro. Toxicol. In Vitro 18:811–819.

Oh, S. M., B. T. Ryu, S. K. Lee, and K. H. Chung. 2007. Antiestrogenic potentials of ortho-PCB congeners by single or complex exposure. Arch. Pharm. Res. 30:199–209.

O'Hara, T. M., and P. R. Becker. 2003. Persistent organic contaminants in Arctic marine mammals. *In* J. G. Vos, G. D. Bossart, M. Fournier, T. O'Shea (eds.), Toxicology of marine mammals, pp. 168–205. Taylor and Francis, New York.

O'Hara, T. M., P. F. Hoekstra, C. Hanns, S. M. Backus, and D. C. G. Muir. 2005. Concentrations of selected persistent organochlorine contaminants in store-bought foods from northern Alaska. Int. J. Circumpolar Health 64:303–313.

O'Hara, T.M., and T. J. O'Shea. 2001. Toxicology. *In* L. A. Dierauf, F. M. D. Gulland (eds.), CRC handbook of marine mammal medicine, 2nd ed, pp. 471–520. CRC Press, Boca Raton, FL.

Oki, C., and S. Atkinson. 2004. Diurnal patterns of cortisol and thyroid hormones in the harbor seal (*Phoca vitulina*) during summer and winter seasons. Gen. Comp. Endocrinol. 136:289–297.

Olesiuk, P. F., M. A. Bigg, and G. M. Ellis. 1990. Life history and population dynamics of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Rep. Int. Whal. Commn. Special Issue 12:209–243.

Olesiuk, P. F., G. M. Ellis, and J. K. B. Ford. 2005. Life history and population dynamics of Northern Resident killer whales (*Orcinus orca*) in British Columbia. Fisheries and Oceans Canada Report no. 2005/045. Nanaimo, BC, Canada.

O'Neill, S. M., and J. E. West. 2009. Marine distribution, life history traits, and the accumulation of polychlorinated biphenyls in Chinook salmon from Puget Sound, Washington. Trans. Am. Fish. Soc. 138:616–632.

O'Neill, S. M., J. E. West, and C. Hoeman. 1998. Spatial trends in the concentration of polychlorinated biphenyls (PCBs) in Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in Puget Sound and factors affecting PCB accumulation: Results from the Puget Sound Ambient Monitoring Program. Puget Sound Research 1998 Proceedings. Puget Sound Water Quality Action Team, Seattle, WA.

O'Neill, S.M., G. M. Ylitalo, and J. E. West. 2014. Energy content of Pacific salmon as prey of northern and southern resident killer whales. Endanger. Species Res. 25:265–281.

O'Neill, S. M., G. M. Ylitalo, J. E. West, J. L. Bolton, C. A. Sloan, and M. M. Krahn. 2006. Regional patterns of persistent organic pollutants in five Pacific salmon species (*Oncorhynchus* spp.) and their contributions to contaminant levels in northern and southern resident killer whales (*Orcinus orca*). Southern Resident Killer Whale Symposium. Seattle, Washington.

O'Shea, T. 1999. Environmental contaminants in marine mammals. *In* J. E. Reynolds, and S. A. Rommel (eds.), Biology of marine mammals. The Smithsonian Institution Press, Washington, D.C.

Pabst, D. A., S. A. Rommel, and W. A. McLellan. 1999. Functional anatomy of marine mammals. *In* J. E. Reynolds and S. A. Rommel (eds.), Biology of marine mammals. The Smithsonian Institution Press, Washington, D.C.

Park, B. K., G. J. Park, Y. R. An, H. G. Choi, G. B. Kim, and H. B. Moon. 2010. Organohalogen contaminants in finless porpoises (*Neophocaena phocaenoides*) from Korean coastal waters: Contamination status, maternal transfer, and ecotoxicological implications. Mar. Pollut. Bull. 60:768–774.

Pellacani, C., S. Tagliaferri, A. Caglieri, M. Goldoni, G. Giordano, A. Mutti, and L. G. Costa. 2014. Synergistic interactions between PBDEs and PCBs in human neuroblastoma cells. Environ. Toxicol. 29:418–427.

Penders, J., E. E. Stobberingh, P. H. M. Savelkoul, and F. G. Wolffs. 2013. The human microbiome as a reservoir of antimicrobial resistance. Front. Microbiol. 4. doi:10.3389/fmicb.2013.00087

Perrin, W. F., and G. P. Donovan. 1984. Report of the workshop. *In* W. F. Perrin, R. L. Brownell, Jr., and D. P. De Master (eds.), Special issue 6: Reproduction in whales, dolphins and porpoises, pp. 97–134 International Whaling Commission, Cambridge.

Perry, J. A., and G. D. Wright. 2013. The antibiotic resistance "mobilome": searching for the link between environment and clinic. Front. Microbiol. 4:138.

Peterson, C. H., S. D. Rice, J. W. Short, D. Esler, J. L. Bodkin, B. E. Ballachey, and D. B. Irons. 2003. Long-term ecosystem response to the *Exxon Valdez* oil spill. Science 302:2082–2086.

Peterson, W. T., R. D. Brodeur, and W. G. Pearcy. 1982. Food-habits of juvenile salmon in the Oregon coastal zone, June 1979. Fish. Bull. 80:841–851.

Pierce, G. J., M. B. Santos, S. Murphy, J. A. Learmonth, A. F. Zuur, E. Rogan, P. Bustamante, F. Caurant, V. Lahaye, V. Ridoux, B. N. Zegers, A. Mets, M. Addink, C. Smeenk, T. Jauniaux, R. J. Law, W. Dabin, A. López, J. M. Alonso Farré, A. F. González, A. Guerra, M. García-Hartmann, R. J. Reid, C. F. Moffat, C. Lockyer, and J. P. Boon. 2008. Bioaccumulation of persistent organic pollutants in female common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from western European seas: Geographical trends, causal factors and effects on reproduction and mortality. Environ. Pollut. 153:401–415.

Plíšková, M., J. Vondracek, R. F. Canton, J. Nera, A. Kocan, J. Petrik, T. Trnovec, T. Sanderson, M. van den Berg, and M. Machala. 2005. Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. Environ. Health Perspect. 113:1277–1284.

Pomeroy, P. P., N. Green, A. J. Hall, M. Walton, K. Jones, and J. Harwood. 1996. Congener-specific exposure of grey seal (*Halichoerus grypus*) pups to chlorinated biphenyls during lactation. Can. J. Fish. Aquat. Sci. 53:1526–1534.

Porterfield, S. P. 2000. Thyroidal dysfunction and environmental chemicals—potential impact on brain development. Environ. Health Perspect. 108 Suppl 3:433–438.

PSAMP (Puget Sound Assessment and Monitoring Program). 2007. Toxic contamination. 2007 Puget Sound Update, pp. 129–194.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, WA.

Quinn, T. P., and K. W. Myers. 2004. Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. Rev. Fish Biol. Fish. 14:421–442.

Raverty, S. A., K. Nielsen, O. Nielsen, M. B. Hanson, and J. K. Gaydos. 2004. Detection of *Brucella* spp. antibodies in postmortem heart blood and antemortem serum of killer whales (*Orcinus orca*) in the Pacific Northwest. Intern. Assoc. Aquat. Anim. Med. Conference Proceedings, Galveston, Texas.

Raverty, S. A., J. K. Gaydos, and J. A. St. Leger. 2014. Killer whale necropsy and disease testing protocol. [Available from http://www.seadocsociety.org/wp-content/uploads/Orca-necropsy-protocol-FINAL-May-15-2014.pdf, accessed May 2016.]

Raverty, S. A., L. D. Rhodes, E. Zabek, A. Eshghi, C. E. Cameron, M. B. Hanson, J. P. Schroeder. In prep. Respiratory microbiome of endangered Southern Resident killer whales and microbiota of surrounding sea surface microlayer in the eastern North Pacific. (Available from L. Rhodes, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle, WA 98112).

Rayne, S., M. G. Ikonomou, P. S. Ross, G. M. Ellis, and L. G. Barrett-Lennard. 2004. PBDEs, PBBs, and PCNs in three communities of free-ranging killer whales (*Orcinus orca*) from the northeastern Pacific Ocean. Environ. Sci. Technol. 38:4293–4299.

Reddy, M. L., J. S. Reif, A. Bachand, and S. H. Ridgway. 2001. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. Sci. Total Environ. 274:171–182.

Reijnders, P. J. 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324:456–457.

Reijnders, P. J. H. 2003. Reproductive and developmental effects of environmental organochlorines on marine mammals. *In* J. G. Vos, G. D. Bossart, M. Fournier, and T. J. O'Shea (eds.), Toxicology of marine mammals, pp. 55–66. Taylor and Francis, New York.

Reijnders, P. J. H., and A. Aguilar. 2002. Pollution and marine mammals. *In* W. F. Perrin, B. Wursig, and J. G. M. Thewissen (eds.), Encyclopedia of marine mammals, pp. 948–957. Academic Press, San Diego, CA.

Reynaud, S., and P. Deschaux. 2006. The effects of polycyclic aromatic hydrocarbons on the immune system of fish: A review. Aquat. Toxicol. 77:229–238.

Rice, S., and A. Moles. 2006. Assessing the potential for remote delivery of persistent organic pollutants to the Kenai River in Alaska. Alaska Fish. Res. Bull. 12:153–157.

Richardson, V. M., D. F. Staskal, D. G. Ross, J. J. Diliberto, M. J. DeVito, and L. Birnbaum. 2008. Possible mechanisms of thyroid hormone disruption in mice by BDE-47, a major polybrominated diphenyl ether congener. Toxicol. Appl. Pharmacol. 226:244–250.

Ridgway, S., and M. Reddy. 1995. Residue levels of several organochlorines in *Tursiops truncatus* milk collected at varied stages of lactation. Mar. Pollut. Bull. 30:609–614.

Rigét, F., A. Bignert, B. Braune, J. Stow, and S. Wilson. 2010. Temporal trends of legacy POPs in Arctic biota, an update. Sci. Total Environ. 408:2874–2884.

Robeck, T. R., S. A. Gearhart, K. J. Steinman, E. Katsumata, J. D. Loureiro, J. K. O'Brien. 2011. In vitro sperm characterization and development of a sperm cryopreservation method using directional solidification in the killer whale (*Orcinus orca*). Theriogenology 76:267–279.

Robeck, T. R., and S. L. Monfort. 2006. Characterization of male killer whale (*Orcinus orca*) sexual maturation and reproductive seasonality. Theriogenology 66:242–250.

Roelens, S. A, V. Beck, G. Aerts, S. Clerens, G. Vanden Bergh, L. Arckens, V. M. Darras, and S. van der Geyten. 2005. Neurotoxicity of polychlorinated biphenyls (PCBs) by disturbance of thyroid hormone-regulated genes. Ann. N. Y. Acad. Sci. 1040:454–456.

Rolland, R. M. 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. J. Wildl. Dis. 36:615–635.

Rolland, R. M., K. E. Hunt, S. D. Kraus,and S. K. Wasser. 2005. Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. Gen. Comp. Endocrinol. 142:308–317.

Rooney, J. P. K. 2007. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology 234:145–156.

Ross, P. S. 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. Hum. Ecol. Risk Assess. 8:277–292.

Ross, P. S., and L. S. Birnbaum. 2003. Integrated human and ecological risk assessment: A case study of persistent organic pollutants (POPs) in humans and wildlife. Hum. Ecol. Risk Assess. 9:303–324.

Ross, P. S., C. M. Couillard, M. G. Ikonomou, S. C. Johannessen, M. Lebeuf, R. W. Macdonald, and G. T. Tomy. 2009. Large and growing environmental reservoirs of deca-BDE present an emerging health risk for fish and marine mammals. Mar. Pollut. Bull. 58:7–10.

Ross, P., R. DeSwart, R. Addison, H. VanLoveren, J. Vos, and A. Osterhaus. 1996. Contaminant-induced immunotoxicity in harbour seals: Wildlife at risk? Toxicology 112:157–169.

Ross, P. S, G. M. Ellis, M. G. Ikonomou, L. G. Barrett-Lennard, and R. F. Addison. 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: Effects of age, sex and dietary preference. Mar. Pollut. Bull. 40:504–515.

Ross, P.S., M. Noël, D. M. Lambourn, N. J. Dangerfield, J. Calambokidis, and S. Jeffries. 2013. Declining concentrations of persistent PCBs, PBDEs, PCDEs, and PCNs in harbor seals (*Phoca vitulina*) from the Salish Sea. Prog. Oceanogr. 155:160–170.

Roubal, W. T., T. K. Collier, and D. C. Malins. 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contam. Toxicol. 5:513–529.

Routti, H., A. Arukwe, B. M. Jenssen, R. J. Letcher, M. Nyman, C. Bäckman, and G. W. Gabrielsen. 2010. Comparative endocrine disruptive effects of contaminants in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Comp. Biochem. Physiol. Part C 152:306–312.

Safe, S., G. Mason, T. Sawyer, T. Zacharewski, M. Harris, C. Yao, B. Keys, K. Farrell, M. Holcomb, D. Davis, L. Safe, J. Piskorska-Pliszczynska, B. Leece, M. A. Denomme, O. Hutzinger, H. Thoma, B. Chittim, and J. Madge. 1989. Development and validation of in vitro induction assays for toxic halogenated aromatic mixtures: A review. Toxicol. Ind. Health 5:757–775.

Salata, G.G., T. L. Wade, J. L. Sericano, J. W. Davis, and J. M. Brooks. 1995. Analysis of Gulf of Mexico bottlenose dolphins for organochlorine pesticides and PCBs. Environ. Pollut. 88:167–175.

Schecter, A., M. Pavuk, O. Päpke, J. J. Ryan, L. Birnbaum, and R. Rosen. 2003. Polybrominated diphenyl ethers (PBDEs) in U.S. mother's milk. Environ. Health Perspect. 111:1723–1729.

Schroeder, J. P., S. Raverty, E. Zabek, C. A. Cameron, A. Eshghi, D. Bain, R. Wood, L. D. Rhodes, and M. B. Hanson. 2010. Investigation into the microbial culture and molecular screening of exhaled breaths of endangered Southern Resident killer whales (SRKW) and pathogen screening of the sea surface microlayer (SML) in Puget Sound. Global Research & Rescue. NOAA Contract #AB133FO9SE4234.

Schwacke, L. H., E. O. Voit, L. J. Hansen, R. S. Wells, G. B. Mitchum, A. A. Hohn, and P. A. Fair. 2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. Environ. Toxicol. Chem. 21:2752–2764.

Schwacke, L. H., E. S. Zolman, B. C. Balmer, S. de Guise, R. C. George, J. Hoguet, A. A. Hohn, J. R. Kucklick, S. Lamb, M. Levin, J. A. Litz, W. E. McFee, N. J. Place, F. I. Townsend, R. S. Wells, and T. K. Rowles. 2012. Anaemia, hypothyroidism and immune suppression associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops truncatus*). Proc. Biol. Sci. 279:48–57.

Schwartz, J. A., B. M. Aldridge, B. L. Lasley, P. W. Snyder, J. L. Stott, and F.C. Mohr. 2004. Chronic fuel oil toxicity in American mink (*Mustela vison*): Systemic and hematological effects of ingestion of a low-concentration of bunker C fuel oil. Toxicol. Appl. Pharmacol. 200(2):146–158.

Sericano, J. L., T. L. Wade, S. T. Sweet, J. Ramirez, and G. G. Lauenstein. 2014. Temporal trends and spatial distribution of DDT in bivalves from the coastal marine environments of the continental United States, 1986–2009. Mar. Pollut. Bull. 81:303–316.

Sharma, R., and T. P. Quinn. 2012. Linkages between life history type and migration pathways in freshwater and marine environments for Chinook salmon, *Oncorhynchus tshawytscha*. Acta Oecol. 41:1–13.

Shaw, S. D., M. L. Berger, D. Brenner, D. O. Carpenter, L. Tao, C. Hong, and K. Kannan. 2008. Polybrominated diphenyl ethers (PBDEs) in farmed and wild salmon marketed in the northeastern United States. Chemosphere 71:1422–1431.

Shaw, S. D., D. Brenner, M. L. Berger, D. O. Carpenter, C. Hong, and K. Kannan. 2006. PCBs, PCDDs/Fs, and organochlorine pesticides in farmed Atlantic salmon from Maine, eastern Canada, and Norway, and wild salmon from Alaska. Environ. Sci. Technol. 40:5347–5354.

Shaw, S. D., D. Brenner, M. L. Berger, F. Fang, C. Hong, R. Storm, D. Hilker, and P. O'Keefe. 2007. Polybrominated diphenyl ethers (PBDEs) in harbor seals from the northwestern Atlantic: Are seals debrominating decaBDE? Organohalogen Compd. 69:829–832.

Shaw, S. D., D. Brenner, C. Hong, B. Bush, and G. M. Shopp. 1999. Low-level exposure to PCBs is associated with immune and endocrine disruption in neonatal harbor seals (*Phoca vitulina*) from the California coast. Organohalogen Compd. 42:11–14.

Shaw, S. D., and K. Kannan. 2009. Polybrominated diphenyl ethers in marine ecosystems of the American continents: Foresight from current knowledge. Rev. Environ. Health 24:157–229.

Shirai, K., T. Sakai, M. Fukuda, and T. Oike. 1998a. A monoclonal antibody against dolphin lymphocytes (6E9) which recognizes bovine MHC class II antigens. J. Vet. Med. Sci. 60:291–293.

Shirai, K., T. Sakai, and T. Oike. 1998b. Molecular cloning of bottle-nosed dolphin (*Tursiops truncatus*) MHC class I cDNA. J. Vet. Med. Sci. 60:1093–1096.

Shoji, Y., Y. Inoue, H. Sugisawa, T. Itou, T. Endo, and T. Sakai. 2001. Molecular cloning and functional characterization of bottlenose dolphin (*Tursiops truncatus*) tumor necrosis factor alpha. Vet. Immunol. Immunopathol. 82:183–192.

Siddiqi, M. A., R. H. Laessig, and K. D. Reed. 2003. Polybrominated diphenyl ethers (PBDEs): New pollutants—Old diseases. Clin. Med. Res. 1:281–290.

Sih, A., A. M. Bell, and J. L. Kerby. 2004. Two stressors are far deadlier than one. Trends Ecol. Evol. 19:274–276.

Silkworth, J. B., and E. M. Grabstein. 1982. Polychlorinated biphenyl immunotoxicity: Dependence on isomer planarity and the Ah gene complex. Toxicol. Appl. Pharmacol. 65:109–115.

Silva, E., N. Rajapakse, and A. Kortenkamp. 2002. Something from "nothing"—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36:1751–1756.

Simms, W., S. Jeffries, M. Ikonomou, and P. S. Ross. 2000. Contaminant-related disruption of vitamin A dynamics in free-ranging harbor seal (*Phoca vitulina*) pups from British Columbia, Canada, and Washington State, U.S.A. Environ. Toxicol. Chem. 19:2844–2849.

Song, L., A. Seeger, and J. Santos-Sacchi. 2005. On membrane motor activity and chloride flux in the outer hair cell: Lessons learned from the environmental toxin tributyltin. Biophys. J. 88:2350–2362.

Sørmo, E. G., B. M. Jenssen, E. Lie, and J. U. Skaare. 2009. Brominated flame retardants in aquatic organisms from the North Sea in comparison with biota from the high Arctic marine environment. Environ. Toxicol. Chem. 28:2082–2090.

Sørmo, E. G., I. Jussi, M. Jussi, M. Braathen, J. U. Skaare, and B. M. Jenssen. 2005. Thyroid hormone status in gray seal (*Halichoerus grypus*) pups from the Baltic Sea and the Atlantic Ocean in relation to organochlorine pollutants. Environ. Toxicol. Chem. 24:610–616.

Spraker, T. R., L. F. Lowry, and K. J. Frost. 1994. Gross necropsy and histopathological lesions found in harbor seals. *In* T. R. Loughlin (ed.), Marine mammals and the *Exxon Valdez*, pp. 281–312. Academic Press, San Diego, CA.

St. Aubin, D. J., and J. R. Geraci. 1989. Adaptive changes in hematologic and plasma chemical constituents in captive beluga whales, *Delphinapterus leucas*. Can. J. Fish. Aquat. Sci. 46:796–803.

St. Aubin, D. J., S. H. Ridgway, R. S. Wells, and H. L. Rhinehart. 1996. Dolphin thyroid and adrenal hormones: circulating levels in wild and semi-domesticated *Tursiops truncatus*, and influence of sex, age, and season. Mar. Mamm. Sci. 12:1–13.

Stockholm Convention on Persistent Organic Pollutants. 2001. Convention Text. Geneva: United Nations Environment Programme. [Available from http://www.pops.int/documents/convtext/convtext_en.pdf, accessed May 2016.]

Stone, D. 2006. Polybrominated diphenyl ethers and polychlorinated biphenyls in different tissue types from Chinook salmon (*Oncorhynchus tshawytscha*). Bull. Environ. Contam. Toxicol. 76:148–154.

Subramanian, A., S. Tanabe, R. Tatsukawa, S. Saito, and N. Miyazaki. 1987. Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of Northwestern North Pacific. Mar. Pollut. Bull. 18:643–646.

Tabuchi, M., N. Veldhoen, N. Dangerfield, S. Jeffries, C. C. Helbing, and P. S. Ross. 2006. PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (*Phoca vitulina*). Environ. Health Perspect. 114:1024–1031.

Tanabe, S., H. Tanaka, K. Maruyama, and R. Tatsukawa. 1981. Ecology and bioaccumulation of *Stenella coeruleoalba*. Elimination of chlorinated hydrocarbons from mother striped dolphins *Stenella coeruleoalba* through parturition and lactation. *In* T. Fujiyama (ed.), Studies on the levels of organochlorine compounds and heavy metals in marine organisms, pp. 115–121. University of the Ryukyus, Okinawa.

Tanabe, S., R. Tatsukawa, K. Maruyama, and N. Miyazaki. 1982. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. Agric. Biol. Chem. 46:1249–1254.

Thomas, P. T., and R. D. Hinsdill. 1978. Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. Toxicol. Appl. Pharmacol. 44:41–51.

Thomas, P. T., and R. D. Hinsdill. 1980. Perinatal PCB exposure and its effect on the immune system of young rabbits. Drug Chem. Toxicol. 3:173–184.

Tilbury, K. L., J. E. Stein, C. A. Krone, R. L. Brownell Jr., S. A. Blokhin, J. L. Bolton, and D. W. Ernest. 2002. Chemical contaminants in juvenlie gray whales (*Eschrichtius robustus*) from a subsistence harvest in Arctic feeding grounds. Chemosphere 47:555–564.

Towers, J. R., G. M. Ellis, and J. K. B. Ford. 2012. Photo-identification catalogue of Bigg's (transient) killer whales from coastal waters of British Columbia, northern Washington, and southeastern Alaska. Canadian Data Report of Fisheries and Aquatic Sciences 1241, 38 p.

Trego, M. L., N. M. Kellar, and K. Danil. 2013. Validation of blubber progesterone concentrations for pregnancy determination in three dolphin species and a porpoise. PLoS ONE 8:e69709

Turusov, V., V. Rakitsky, and L. Tomatis. 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. Environ. Health Perspect. 110:125–128.

Van Bressem, M. F., K. Van Waerebeek, and J. A. Raga. 1999. A review of virus infections of cataceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. Dis. Aquat. Organ. 38:53–65.

Vandenberg, L. N., T. Colborn, T. B. Hayes, J. J. Heindel, D. R. Jacobs, D. Lee, T. Shioda, A. M. Soto, F. S. vom Saal, W. V. Welshons, R. T. Zoeller, and J. P. Myers. 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33:378–455.

Van den Brink, N. W., E. M. de Ruiter-Dijkman, S. Broekhuizen, P. J. H. Reijnders, and A. T. C. Bosveld. 2000. Polychlorinated biphenyls pattern analysis: Potential nondestructive biomarker in vertebrates for exposure to cytochrome P450-inducing organochlorines. Environ. Toxicol. Chem. 19:575–581.

Van de Vijver, K. I., P. T. Hoff, K. Das, W. Van Dongen, E. L. Esmans, T. Jauniaux, J. Bouquegneau, R. Blust, and W. de Coen W. 2003. Perfluorinated chemicals infiltrate ocean waters: Link between exposure levels and stable isotope ratios in marine mammals. Environ. Sci. Technol. 37:5545–5550.

Varanasi, U., D. W. Brown, T. Hom, D. G. Burrows, C. A. Sloan, F. J. Field, J. E. Stein, K. L. Tilbury, B. B. McCain, and S Chan. 1993a. Supplemental information concerning a survey of Alaskan subsistence fish, marine mammal, and invertebrate samples collected 1989–91 for exposure to oil spilled from the *Exxon Valdez*, vol. 2. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-13, 173 p.

Varanasi, U., E. Casillas, M. R. Arkoosh, T. Hom, D. A. Misitano, D. W. Brown, S. L. Chan, T. K. Collier, B. B. McCain, and J. E. Stein. 1993b. Contaminant exposure and associated biological effects in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from urban and nonurban estuaries of Puget Sound, WA. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-8, 112 p.

Varanasi, U., J. E. Stein, and M. Nishimoto. 1989. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. *In* U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment, pp. 94–149. CRC Press, Boca Raton, FL.

Veldhoen, N., M. G. Ikonomou, C. Dubetz, N. Macpherson, T. Sampson, B. C. Kelly, and C. C. Helbing. 2010. Gene expression profiling and environmental contaminant assessment of migrating Pacific salmon in the Fraser River watershed of British Columbia. Aquat. Toxicol. 97:212–225.

Vélez-Espino, L. A., J. K. B. Ford, H. A. Araujo, G. Ellis, C. K. Parken, and K. C. Balcomb. 2014a. Comparative demography and viability of Northeastern Pacific resident killer whale populations at risk. Fisheries and Oceans Canada Report no. 3084, 66 p. Nanaimo, BC, Canada.

Vélez-Espino, L. A., J. K. B. Ford, H. A. Araujo, G. Ellis, C. K. Parken, and R. Sharma. 2014b. Relative importance of chinook salmon abundance on resident killer whale population growth and viability. Aquat. Conserv. 25:756–780. doi:10.1002/aqc.2494

Venn-Watson, S., K. M. Colegrove, J. Litz, M. Kinsel, K. Terio, J. Saliki, S. Fire, R. Carmichael, C. Chevis, W. Hatchett, J. Pitchford, M. Tumlin, C. Field, S. Smith, R. Ewing, D. Fauquier, G. Lovewell, H. Whitehead, D. Rotstein, W. McFee, E. Fougeres, and T. Rowles. 2015. Adrenal gland and lung lesions in Gulf of Mexico common bottlenose dolphins (*Tursiops truncatus*) found dead following the *Deepwater Horizon* oil spill. PLoS ONE 10(5):e0126538. doi:10.1371/journal.pone.0126538

Viberg, H., A. Fredriksson, and P. Eriksson. 2003. Neonatal exposure to polybrominated diphenyl ether (PBDE-153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. Toxicol. Appl. Pharmacol. 192:95–106.

Viberg, H., N. Johansson, A. Fredriksson, J. Eriksson, G. Marsh, and P. Eriksson. 2006. Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. Toxicol. Sci. 92:211–218.

Wania, F., and D. Mackay. 1996. Tracking the distribution of persistent organic pollutants. Environ. Sci. Technol. 30:390A–396A.

Ward, E. J., M. J. Ford, R. G. Kope, J. K. B. Ford, L. A. Velez-Espino, C. K. Parken, L. W. LaVoy, M. B. Hanson, and K. C. Balcomb. 2013. Estimating the impacts of Chinook salmon abundance and prey removal by ocean fishing on Southern Resident killer whale population dynamics. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-123, 71 p.

Ward, E. J., E. E. Holmes, and K. C. Balcomb. 2009. Quantifying the effects of prey abundance on killer whale reproduction. J. Appl. Ecol. 46:632–640.

Wasser, S., J. I. Lundin, R. K. Booth, L. Seely, D. A. Giles, J. A. Hempelmann, K. M. Parsons, and M. Ford. In prep. Poor nutrition, vessel effects, and reproductive failure in Southern Resident killer whales. (Available from S. Wasser, Center for Conservation Biology, Johnson Hall 231, Box 351800, University of Washington, Seattle, WA 98195.)

Weisskopf, M. G., H. A. Anderson, L. P. Hanrahan, and C. Great Lakes. 2003. Decreased sex ratio following maternal exposure to polychlorinated biphenyls from contaminated Great Lakes sport-caught fish: A retrospective cohort study. Environ. Health 2:2.

Weitkamp, L. A. 2010. Marine distributions of Chinook salmon from the west coast of North America determined by coded wire tag recoveries. Trans. Am. Fish. Soc. 139:147–170.

Wells, R. S., V. Tornero, A. Borrell, A. Aguilar, T. K. Rowles, H. L. Rhinehart, S. Hofmann, W. M. Jarman, A. A. Hohn, and J. C. Sweeney. 2005. Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Sci. Total Environ. 349:106–119.

West, J., S. O'Neill, J. Lanksbury, G.M. Ylitalo, and S. Redman. 2011. Current conditions, time trends and recovery targets for toxic contaminants in Puget Sound fish: The Toxics in Fish Dashboard Indicator. [Available from http://wdfw.wa.gov/publications/01364/, accessed May 2016.]

West, J., S. O'Neill, G. Lippert,and S. Quinnell. 2001. Toxic contaminants in marine and anadramous fishes from Puget Sound, Washington. Olympia, WA: Results of the Puget Sound Ambient Monitoring Program Fish Component, 1989–1999. Washington Department of Fish and Wildlife Report no. WDFW 765.

West, J. E., S. M. O'Neill, and G. M. Ylitalo. 2008. Spatial extent, magnitude, and patterns of persistent organochlorine pollutants in Pacific herring (*Clupea pallasi*) populations in the Puget Sound (USA) and Strait of Georgia (Canada). Sci. Total Environ. 394:369–378.

WHO (World Health Organization). 2010. Exposure to dioxins and dioxin-like substances: A major public health concern. Preventing Disease Through Healthy Environments, Geneva, Switzerland.

Wiles, G. J. 2004. Washington State status report for the killer whale. Washington Department of Fish and Wildlife, 106 p. Olympia, WA.

Wolff, M. S., D. Camann, M. Gammon, and S. D. Stellman. 1997. Proposed PCB congener groupings for epidemiological studies. Environ. Health Perspect. 105:13–14.

Wolkers, H., P. J. Corkeron, S. M. Van Parijs, T. Simila, and B. Van Bavel. 2007. Accumulation and transfer of contaminants in killer whales (*Orcinus orca*) from Norway: Indications for contaminant metabolism. Environ. Toxicol. Chem. 26:1582–1590.

Ylitalo, G. M., R. W. Baird, G. K. Yanagida, D. L. Webster, S. J. Chivers, J. L. Bolton, G. S. Schorr, and D. J. McSweeney. 2009. High levels of persistent organic pollutants measured in blubber of island-associated false killer whales (*Pseudorca crassidens*) around the main Hawaiian Islands. Mar. Pollut. Bull. 58:1922–1952.

Ylitalo, G. M., C. O. Matkin, J. Buzitis, M. M. Krahn, L. L. Jones, T. Rowles, and J. E. Stein. 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, Alaska. Sci. Total Environ. 281:183–203.

Ylitalo, G. M., M. Myers, B. S. Stewart, P. K. Yochem, R. Braun, L. Kashinsky, D. Boyd, G. A. Antonelis, S. Atkinson, A. A. Aguirre, and M. M. Krahn. 2008. Organochlorine contaminants in endangered Hawaiian monk seals from four subpopulations in the northwestern Hawaiian islands. Mar. Pollut. Bull. 56:231–244.

Ylitalo, G. M., J. E. Stein, T. Horn, L. L. Johnson, K. L. Tilbury, A. J. Hall, T. Rowles, D. Greig, L. J. Lowenstine, and F. M. Gulland. 2005. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). Mar. Pollut. Bull. 50:30–39.

Yordy, J. E., M. A. Mollenhauer, R. M. Wilson, R. S. Wells, A. Hohn, J. Sweeney, L. H. Schwacke, T. K. Rowles, J. R. Kucklick, and M. M. Peden-Adams. 2010a. Complex contaminant exposure in cetaceans: A comparative e-screen analysis of bottlenose dolphin blubber and mixtures of four persistent organic pollutants. Environ. Toxicol. Chem. 29:2143–2153.

Yordy, J. E., D. A. Pabst, W. A. McLellan, R. S Wells, T. K. Rowles, and J. R. Kucklick. 2010b. Tissue-specific distribution and whole-body burden estimates of persistent organic pollutants in the bottlenose dolphin (*Tursiops truncatus*). Environ. Toxicol. Chem. 29:1263–1273.

Yordy, J. E., R. S. Wells, B. C. Balmer, L. H. Schwacke, T. K. Rowles, and J. R. Kucklick. 2010c. Partitioning of persistent organic pollutants between blubber and blood of wild bottlenose dolphins: Implications for biomonitoring and health. Environ. Sci. Technol. 44:4789–4795.

Zakharov, V. M., and A. V. Yablokov. 1990. Skull asymmetry in the Baltic gray seal: Effects of environmental pollution. Ambio 19:266–269.

Zamon, J. E., T. J. Guy, K. C. Balcomb, and D. K. Ellifrit. 2007. Winter observations of Southern Resident killer whales (*Orcinus orca*) near the Columbia River plume during the 2005 spring Chinook salmon (*Oncorhynchus tshawytscha*) spawning migration. Northwest Nat. 88:193–198.

Zhou, T., D. G. Ross, M. J. DeVito, and K. M. Crofton. 2001. Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. Toxicol. Sci. 61:76–82.

Zhou, T., M. M. Taylor, M. J. DeVito, and K. M. Crofton. 2002. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. Toxicol. Sci. 66:105–116.

Appendix A:
Infectious Diseases Reported for Killer Whales

In addition to those caused by specific, known pathogens, infectious diseases not attributable to identified or specific agents have been commonly cited for captive animals. For example, pneumonia, abscesses, and septicemia are frequently observed without identification of specific etiologic agents (e.g., see Ridgway 1979, Greenwood and Taylor 1985, and this list of deceased killer whales[4]). Although environmental conditions for captive animals differ considerably, clinical observations demonstrate the potential for killer whales to be hosts to a multitude of infectious and opportunistic agents, including many that also have been recovered from sympatric marine mammals and humans.

Infectious disease epidemics have not yet been observed among wild killer whales. Mass strandings of killer whales appear to be related more to entrapment by physical conditions (e.g., ice or outgoing tide) or deliberate hunting activity than to diseases (e.g., Uni et al. 2005, Lopez and Lopez 1985), although the possible contribution of algal toxins, seismic effects, sonar, climate change, or other underlying factors cannot be discounted. Network theory modeling predicts that the transient killer whales of the northeast Pacific are highly vulnerable to an infectious disease epidemic, based on population size, close social structure, and high degree of behavioral interaction within social groups (Guimaraes et al. 2007). The Southern Resident killer whale population is much smaller than the model population, has a narrower geographic distribution, and probably exhibits a similar highly interactive social network, suggesting that it is also at high risk for infectious disease outbreaks.

[4] http://www.angelfire.com/nb/orca/dead.html

Table A-1. Known or potential pathogens identified in killer whales. The method of detection and whale origin are included. KEY: EM = electron microscopy; Antibodies = pathogen-reactive antibodies; Culture = in vitro culture; PCR = pathogen-specific polymerase chain reaction; Microscopy = histology or whole mount microscopy.

Pathogens	Method(s) of Detection	Wild or Captive	Reference(s)
Viruses			
Cetacean pox-like virus (Orthopoxvirus)	EM	Wild	Van Bressem et al. (1999)
Cutaneous papillomavirus	EM	Captive	Bossart et al. (1996), Bossart et al. (2002)
Hepatitis B-like virus	Antibodies	Captive	Bossart et al. (1990)
Influenza (suspected)	Symptoms	Captive	Ridgway (1979)
Morbillivirus	Antibodies	Wild (live capture)	S. Raverty (pers. commun.)[a]
West Nile virus	Microarray, RTPCR, immunohistology	Captive	St. Leger et al. (2011)
Bacteria			
Aeromonas sp.	Culture	Wild	Schroeder et al. (2010)
Brucella spp.	Antibodies, culture, PCR	Wild	Jepson et al. (1997), Raverty et al. (2004)
Burkholderia pseudomallei	Culture	Captive	Hicks et al. (2000)
Burkholderia sp.	Culture	Wild	Schroeder et al. (2010)
Clostridium perfringens	Culture	Wild	Schroeder et al. (2010)
Corynebacterium ulcerans	Culture	Wild	Seto et al. (2008)
Edwardsiella tarda	Culture	Wild	Colegrove et al. (2010)
Erysipelothrix rhusiopathiae	Culture	Wild, captive	Bossart and Eimstad (1988)
Kocuria sp.	Culture	Wild	Schroeder et al. (2010)
Mycoplasma spp. (Mollicutes)	PCR	Wild	Schroeder et al. (2010)
Nocardia farcinica, N. asteroids, N. otitidiscaviarum	Culture and/or PCR	Captive	Sweeney and Ridgway (1975), Lamere et al. (2009)
Pseudomonas aeruginosa	Culture	Captive, wild capture and introduction	S. Raverty (pers. commun.)[a]
Pseudomonas sp.	Culture	Wild	Schroeder et al. (2010)
Rothia dentocariosa	Culture	Wild	Schroeder et al. (2010)
Salmonella enterica serovar Heidelberg	Culture	Wild	Schroeder et al. (2010)
Salmonella enterica serovar Newport	Culture	Wild	Colegrove et al. (2010)
Staphylococcus aureus	Culture	Wild	Power and Murphy (2002)
Staphylococcus capitis	Culture	Wild	Schroeder et al. (2010)
Staphylococcus cohnii cohnii	Culture	Wild	Schroeder et al. (2010)
Staphylococcus epidermidis	Culture	Wild	Schroeder et al. (2010)
Staphylococcus lugdunensis	Culture	Wild	Schroeder et al. (2010)
Staphylococcus sp.	Culture	Wild	Schroeder et al. (2010)
Staphylococcus warneri	Culture	Wild	Schroeder et al. (2010)
Stenotrophomonas sp.	Culture	Wild	Schroeder et al. (2010)

[a] Raverty, S. 2014. Pers. commun. Ministry of Agriculture and Lands, Animal Health Center, Abbotsford, BC, Canada.

Table A-1 continued. Known or potential pathogens identified in killer whales. The method of detection and whale origin are included. KEY: EM = electron microscopy; Antibodies = pathogen-reactive antibodies; Culture = in vitro culture; PCR = pathogen-specific polymerase chain reaction; Microscopy = histology or whole mount microscopy.

Pathogens	Method(s) of Detection	Wild or Captive	Reference(s)
Bacteria			
Streptococcus sp., alpha-hemolytic	Culture	Wild	Schroeder et al. (2010)
Streptococcus sp., beta-hemolytic	Culture	Captive	Greenwood and Taylor (1985)
Vibrio alginolyticus	Culture	Wild	Schroeder et al. (2010)
Fungi			
Alternaria sp.	Culture	Wild	Schroeder et al. (2010)
Aspergillus fumigatus	Culture	Captive	Reidarson et al. (1999)
Candida albicans	Culture	Captive	Sweeney and Ridgway (1975), Ridgway (1979), Greenwood and Taylor (1985)
Cladosporidium sp.	Culture	Wild	Schroeder et al. (2010)
Cunninhamella bertholletiae	Culture	Captive	Abdo et al. (2012)
Phoma sp.	Culture	Wild	Schroeder et al. (2010)
Saksenaea vasiformis		Captive	Reidarson et al. (1999), Robeck and Dalton (2002)
Parasites: Acanthocephala			
Bolbosoma niponicum	Unknown	Unknown	Heptner et al.(1976)
Bolbosoma physeteris	Microscopy	Wild	Gibson et al. (1998)
Parasites: Cestoda			
Diphyllobothrium polyrugosum	Microscopy	Wild	Gibson et al. (1998)
Trigonocotyle spasskyi	Unknown	Unknown	Daily and Brownell (1972)
Parasites: Nematoda			
Anisakis simplex	Microscopy	Wild	Gibson et al. (1998)
Anisakis pacificus	Unknown	Unknown	Heptner et al. (1976)
Parasites: Protozoa			
Kyaroikeu cetarius	Microscopy	Unknown	Sniezek et al. (1995)
Neospora caninum	Antibodies	Wild	Omata et al. (2006)
Sarcosystis sp.	PCR	Wild	Gibson et al. (2011), S. Raverty (pers. commun.)[a]
Toxoplasma gondii	Antibodies, PCR	Wild, captive	Murata et al. (2004), Gibson et al. (2011)
Parasites: Trematoda			
Campula sp.	Microscopy	Wild	Gibson et al. (1998)
Fasciola skrjabini	Unknown	Unknown	Daily and Brownell (1972)
Leucasiella subtilla	Unknown	Unknown	Heptner et al. (1976)
Oschmarinella albamarina	Microscopy	Wild	Gibson et al. (1998)

[a] Raverty, S. 2014. Pers. commun. Ministry of Agriculture and Lands, Animal Health Center, Abbotsford, BC, Canada.

References: Appendix A

Abdo, W., Y. Kakizoe, M. Ryono, S. R. Dover, H. Fukushi, H. Okuda, R. Kano, T. Shibahara, E. Okada, H. Sakai, and T. Yanai. 2012. Pulmonary zygomycosis with *Cunninghamella bertholletiae* in a killer whale (*Orcinus orca*). J. Comp. Pathol. 147:94–99.

Bossart, G. D., T. A. Brawner, C. Cabal, M. Kuhns, E. A. Eimstad, J. Caron, M. Trimm, and P. Bradley. 1990. Hepatitis B-like infection in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). J. Am. Vet. Med. Assoc. 196:127–130.

Bossart, G. D., C. Cray, J. L. Solorazano, S. J. Decker, L. H. Cornell, and N. H. Altman. 1996. Cutaneous papillomaviral-like papillomatosis in a killer whale (*Orcinus orca*). Mar. Mamm. Sci. 12:274–281.

Bossart, G. D., S. J. Decker, and R. Y. Ewing. 2002. Cytopathology of cutaneous viral papillomatosis in the killer whale (*Orcinus orca*). *In* C. J. Pfeiffer (ed.), Molecular and cell biology of marine mammals, pp. 213–224. Krieger Publishing Company, Melbourne, FL.

Bossart, G. D., and E. Eimstad. 1988. *Erysipelothrix* vesicular glossitis in a killer whale (*Orcinus orca*). J. Zoo Anim. Med. 19:42–47.

Colegrove, K. M., J. A. St. Leger, S. Raverty, S. Jang, M. Berman-Kowalewski, and J. K. Gaydos. 2010. Salmonella Newport omphaloarteritis in a stranded killer whale (*Orcinus orca*) neonate. J. Wildl. Dis. 46:1300–1304.

Daily, M. D., and R. L. Brownell, Jr. 1972. A checklist of marine mammal parasites. *In* S. H. Ridgway (ed.), Mammals of the sea: Biology and medicine, pp. 528–589. Charles C. Thompson, Springfield, IL.

Gibson, A. K., S. Raverty, D. M. Lambourn, J. Huggins, S. L. Magargal, and M. E. Grigg. 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. PLoS Negl. Trop. Dis. 5:e1142.

Gibson, D. I., E. A. Harris, R. A. Bray, P. D. Jepson, T. Kuiken, J. R. Baker, and V. R. Simpson. 1998. A survey of the helminth parasites of cetaceans stranded on the coast of England and Wales during the period 1990–1994. J. Zool. 244:563–574.

Greenwood, A. G., and D. C. Taylor. 1985. Captive killer whales in Europe. Aquat. Mamm. 1:10–12.

Guimaraes, P. R., Jr., M. A. de Menezes, R. W. Baird, D. Lusseau, P. Guimaraes, and S. F. dos Reis. 2007. Vulnerability of a killer whale social network to disease outbreaks. Phys. Rev. E. Stat. Nonlin. Soft Matter Phys. 76:042901.

Heptner, V. G., K. K. Chapskii, V. A. Arsen'ev, and V. E. Sokolov. 1976. Pinnipedia and Odontoceti. Mammals of the Soviet Union, vol. 2, part 3. Vysshaya Shkola Publishers, Moscow, Russia.

Hicks, C. L., R. Kinoshita, and P. W. Ladds. 2000. Pathology of melioidosis in captive marine mammals. Aust. Vet. J. 78:193–195.

Jepson, P. D., S. Brew, A. P. MacMillan, J. R. Baker, J. Barnett, J. K. Kirkwood, T. Kuiken, I. R. Robinson, and V. R. Simpson. 1997. Antibodies to *Brucella* in marine mammals around the coast of England and Wales. Vet. Rec. 141:513–515.

Lamere, S. A., J. A. St Leger, M. D. Schrenzel, S. J. Anthony, B. A. Rideout, and D. R. Salomon. 2009. Molecular characterization of a novel gammaretrovirus in killer whales (*Orcinus orca*). J. Virol. 83:12956–12967.

Lopez, J. C., and D. Lopez. 1985. Killer whales (*Orcinus orca*) of Patagonia, and their behavior of intentional stranding while hunting nearshore. J. Mammal. 66:181–183.

Murata, K., K. Mizuta, K. Imazu, F. Terasawa, M. Taki, and T. Endoh. 2004. The prevalence of *Toxoplasma gondii* antibodies in wild and captive cetaceans from Japan. J. Parasitol. 90:896–898.

Omata, Y., Y. Umeshita, M. Watarai, M. Tachibana, M. Sasaki, K. Murata, and T. K. Yamada. 2006. Investigation for presence of *Neospora caninum*, *Toxoplasma gondii* and *Brucella*-species infection in killer whales *(Orcinus orca*) mass-stranded on the coast of Shiretoko, Hokkaido, Japan. J. Vet. Med. Sci. 68:523–526.

Power E., and S. Murphy. 2002. Staphylococcus septicaemia in a killer whale. Vet. Rec. 150(26):819.

Raverty, S. A., K. Nielsen, O. Nielsen, M. B. Hanson, and J. K. Gaydos. 2004. Detection of *Brucella* spp. antibodies in postmortem heart blood and antemortem serum of killer whales (*Orcinus orca*) in the Pacific Northwest. *In* Proceedings of the 35th Conference of the International Association of Aquatic Animal Medicine, pp. 114-115. Galveston, TX.

Reidarson, T. H., J. F. McBain, L. M. Dalton, and M. G. Rinaldi. 1999. Diagnosis and treatment of fungal infections in marine mammals. *In* M. E. Fowler and R. E. Miller (eds.), Zoo and wild animal medicine: Current therapy, pp. 478–485. W. B. Saunders Company, Philadelphia, PA.

Ridgway, S. H. 1979. Reported causes of death of captive killer whales (*Orcinus orca*). J. Wildl. Dis. 15:99–104.

Robeck, T. R., and L. M. Dalton. 2002. *Saksenaea vasiformis* and *Apophysomyces elegans* zygomycotic infections in bottlenose dolphins (*Tursiops truncatus*), a killer whale (*Orcinus orca*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). J. Zoo. Wildl. Med. 33:356–366.

Schroeder, J. P., S. Raverty, E. Zabek, C. A. Cameron, A. Eshghi, D. Bain, R. Wood, L. D. Rhodes, and M. B. Hanson. 2010. Investigation into the microbial culture and molecular screening of exhaled breaths of endangered Southern Resident killer whales (SRKW) and pathogen screening of the sea surface microlayer (SML) in Puget Sound. Global Research & Rescue. NOAA Contract #AB133FO9SE4234.

Seto, Y., T. Komiya, M. Iwaki, T. Kohda, M. Mukamoto, M. Takahashi, and S. Kozaki. 2008. Properties of corynephage attachment site and molecular epidemiology of *Corynebacterium ulcerans* isolated from humans and animals in Japan. Jpn. J. Infect. Dis. 61:116–122.

Sniezek, J. H., D. W. Coats, and E. B. Small. 1995. *Kyaroikeus cetarius* N-G, N-Sp: A parasitic ciliate from the respiratory tract of odonticete cetacea. J. Eukaryot. Microbiol. 42:260–268.

St. Leger, J., G. Wu, M. Anderson, L. Dalton, E. Nilson, and D. Wang. 2011. West Nile Virus infection in killer whale, Texas, USA, 2007. Emerg. Infect. Dis. 17:1531–1533.

Sweeney, J. C., and S. H. Ridgway. 1975. Common diseases of small cetaceans. J. Am. Vet. Med. Assoc. 167:533–540.

Uni, Y., M. Tazawa, and Y. Masuda. 2005. Mass stranding of killer whale in pack ice at Aidomari, Rause, Hokkaido (Preliminary report). [Available from http://www.h6.dion.ne.jp/~unisan/data/imc9_akw/imc9_akw.html, accessed 10 November 2016.]

Van Bressem M. F., K. Van Waerebeek, and J. A. Raga. 1999. A review of virus infections of cataceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. Dis. Aquat. Organ. 38:53–65.

Appendix B: Noninfectious Diseases Reported for Killer Whales

There has been one reported observation of an apparent genetic disease, Chediak-Higashi syndrome, an autosomal recessive trait (Ridgway 1979). This syndrome presents with a loss of cutaneous pigmentation, and has been reported to occur in at least six divergent species, including humans. Notably, affected individuals are prone to recurrent infections and low platelet counts. The whale reported by Ridgway (1979) died more than 2 years after capture, but the proximal cause of death was not cited.

Systemic changes that are not clearly attributable to infection have been reported in killer whales. For example, extensive atherosclerotic change with thrombi in the aorta and carotid artery was reported without an associated infection or etiologic agent (Roberts et al. 1965). This condition is commonly observed in beluga and other deep diving cetaceans, and may be an adaptive physiologic response, rather than a pathology.

Because killer whales are relatively long-lived, neoplasms are likely to occur, but few have been observed. A diagnosis of Hodgkin's disease in a captive whale was based on cytologic criteria, and whether the disease was fatal is unknown (Yonezawa et al. 1989). Reported skin papillomas are likely to have a viral origin, although this has not always been confirmed (Geraci et al. 1987, Bossart et al. 1996). Genital papillomas have also been observed (Greenwood et al. 1974), but whether the lesions were likely to interfere with reproductive activity or fecundity was not noted.

Recently, an endogenous gammaretroviral provirus was cloned from the killer whale genome, which was the first retrovirus isolated from a cetacean (Lamere et al. 2009). Although some members of this genus are oncogenic (e.g., murine leukemia virus, feline leukemia virus), many are found in multiple host species without associated pathology. The lack of evidence of viral RNA suggested the killer whale provirus was not disease-causing (Lamere et al. 2009).

References: Appendix B

Bossart, G. D., C. Cray, J. L. Solorazano, S. J. Decker, L. H. Cornell, and N. H. Altman. 1996. Cutaneous papillomaviral-like papillomatosis in a killer whale (*Orcinus orca*). Mar. Mamm. Sci. 12:274–281.

Geraci, J. R., N. C. Palmer, and D. J. St. Aubin. 1987. Tumors in cetaceans: Analysis and new findings. Can. J. Fish. Aquat. Sci. 44:1289–1300.

Greenwood, A. G., R. J. Harrison, and H. W. Whitting. 1974. Functional and pathological aspects of the skin of marine mammals. *In* R. J. Harrison (ed.), Functional anatomy of marine mammals, vol. 2, pp. 73–110. Academic Press, London, U.K.

Lamere, S. A., J. A. St. Leger, M. D. Schrenzel, S. J. Anthony, B. A. Rideout, and D. R. Salomon. 2009. Molecular characterization of a novel gammaretrovirus in killer whales (*Orcinus orca*). J. Virol. 83:12956–12967.

Ridgway, S. H. 1979. Reported causes of death of captive killer whales (*Orcinus orca*). J. Wildl. Dis. 15:99–104.

Roberts, J. C., R. C. Boice, R. L. Brownell, Jr., and D. H. Brown. 1965. Spontaneous atherosclerosis in Pacific toothed and baleen whales. *In* J. C. Roberts and R. Strauss (eds.), Comparative atherosclerosis, pp. 151–155. Harper & Row, New York.

Yonezawa, M., H. Nakamine, T. Tanaka, and T. Miyaji. 1989. Hodgkin's disease in a killer whale (*Orcinus orca*). J. Comp. Pathol. 100:203–207.

Appendix C: Infectious Diseases of Sympatric Marine Mammals

Identification of emerging infectious diseases among marine mammals is expanding with more focused research on nearshore and stressed populations, a heightened awareness of factors that may contribute to epidemics (Van Bressem et al. 2009), and the advancement of diagnostic tools. Sympatric marine mammals are potential reservoirs and vectors for infectious agents that have not yet been isolated from killer whales. Table C-1 presents a listing of known and high-probability or putative pathogens of sympatric cetaceans and pinnipeds, and the target tissues for infection.

Notably, many of these listed agents can infect divergent vertebrate hosts, including humans, birds, invertebrates, and terrestrial animals. Stormwater runoff and effluents from urban or agricultural activities are recognized sources of these and other pathogens that can infect marine mammals, resulting in increased concentrations in marine receiving waters (Arnone and Walling 2007). For example, Conrad et al. (2005) show that the source of toxoplasmosis and sarcocystosis, a significant source of morbidity and mortality for sea otter along the California coast, is most likely freshwater runoff carrying oocysts shed by terrestrial animals, particularly felids (toxoplasmosis) and opossums (sarcocystosis). Pathogens capable of infecting multispecies hosts, such as *T. gondii*, are a particular threat for endangered populations.

In the Pacific Northwest, the appearance of *Cryptococcus neoformans gattii* (now *Cryptococcus gattii*) in free-ranging harbor porpoise, Dall's porpoise (*Phocoenoides dalli*), and a solitary Pacific white-sided dolphin (*Lagenorhynchus obliquidens)* as part of a multispecies (including humans; Upton et al. 2007), large-scale marine and terrestrial outbreak of cryptococcosis (Stephen et al. 2002), has stimulated concern about the extent and prognosis for this emerging disease (MacDougall et al. 2007). For humans, both immunocompromised and immunocompetent individuals have developed disease (Datta et al. 2009), underscoring the inherent virulence of this pathogen.

Table C-1. Known and high-probability or putative pathogens affecting sympatric species of cetaceans and pinnipeds. The target tissues for infection are indicated by an *x*. Target tissues included as *Other* are the liver, lymph nodes, kidney, and placenta.

Pathogens	Target Tissues					Host Species[a]	Reference(s)
	Enteric	Skin	Lung	Neural	Other		
Viruses							
Influenza A virus	x		x		x	*Ph*	Danner et al. (1998)
Morbillivirus (cetacean, phocine)	x	x	x	x	x	*Pv*	Duignan et al. (1995)
Norovirus/Calicivirus	x					*Cu, Ej, Er, Ma, Ta, Zc*	Smith et al. (1998)
Rotavirus			x			*Zc*	S. Raverty (pers. commun.)[b]
Bacteria							
Brucella spp.					x	*Pv*	Godfroid et al. (2005), Lambourn et al. (2006)
Campylobacter jejuni	x					*Ma*	Stoddard et al. (2008)
Clostridium difficile	x					*Pv*	Raverty et al. (2005)
Clostridium perfringens	x				x	*Tt*	Buck et al. (1987)
Coxiella burnetii						*Ej, Pv*	Kersh et al. (2010), Lapointe et al. (1999)
Edwardsiella tarda						*Pd, Pp*	Raverty et al. (2005)
Escherichia coli	x	x	x	x	x	*Pv*	Raverty et al. (2005)
Klebsiella pneumoniae			x			*Zc*	Jang et al. (2010)
Leptospira interrogans, *L. kirschneri*				x	x	*Ma, Zc*	Cameron et al. (2008), Lloyd-Smith et al. (2007), Raverty et al. (2005)
Pasteurella hemolytica					x	*Pv*	Steiger et al. (1989)
Plesiomonas shigelloides	x					*Pp, Zc*	Jagger et al. (2000)
Pseudomonas spp.			x		x	*Pv*	Steiger et al. (1989), Raverty et al. (2005)
Salmonella spp.	x		x			*Ma, Pv, Zc*	Gilmartin et al. (1979), Thornton et al. (1998), Stoddard et al. (2008)
Staphylococcus aureus		x				*Ta*	Palmer et al. (1991)
Staphylococcus spp.					x	*Pp, Zc*	Siebert et al. (2002), Fravel and Evans (2008)
Streptococcus spp.				x	x	*El, Pv*	Steiger et al. (1989), Imai et al. (2009)
Vibrio spp.	x					*Pd, Pv, Tt*	Schroeder et al. (1985), Buck and Spotte (1986), Fujioka et al. (1988), Raverty et al. (2005)

[a] Host species are abbreviated as follows: *Bm, Balaena mysticetus* (bowhead whale); *Cu, Callorhinus ursinus* (Northern fur seal); *Dl, Delphinapterus leucas* (beluga whale); *Ej, Eumetopias jubatus* (Stellar sea lion); *El, Enhydra lutris* (sea otter); *Er, Eschrichtius robustus* (gray whale); *Kb, Kogia breviceps* (pygmy sperm whale); *Lo, Lagenorhynchus obliquidens* (Pacific white-sided dolphin); *Ma, Mirounga angustirostris* (Northern elephant seal); *Or, Odobenus rosmarus* (walrus); *Pd, Phocoenoides dalli* (Dall's porpoise); *Ph, Phoca hispida* (ringed seal); *Pp, Phocoena phocoena* (harbour porpoise); *Pv, Phoca vitulina* (harbor seal); *Ta, Tursiops aduncus* (Indo-Pacific bottlenose dolphin); *Tt, Tursiops truncatus* (common bottlenose dolphin); *Zc, Zalophus californianus* (California sea lion).
[b] Raverty, S. 2014. Pers. commun. Ministry of Agriculture and Lands, Animal Health Center, Abbotsford, BC, Canada.

Table C-1 continued. Known and high-probability or putative pathogens affecting sympatric species of cetaceans and pinnipeds. The target tissues for infection are indicated by an *x*. Target tissues included as *Other* are the liver, lymph nodes, kidney, and placenta.

Pathogens	Target Tissues					Host Species[a]	Reference(s)
	Enteric	Skin	Lung	Neural	Other		
Protozoa							
Cryptosporidium spp.	x					*Bm, Dl, Ph, Zc*	Deng et al. (2000), Hughes-Hanks et al. (2005)
Giardia spp.	x					*Bm, Dl, Ph, Zc*	Deng et al. (2000), Hughes-Hanks et al. (2005)
Neospora canium, *N. canium*-like	x			x		*El*	S. Raverty (pers. commun.)[b]
Sarcocystis spp., *S. canis*, *S. neurona*				x	x	*Ej, El, Pv*	Lapointe et al. (1998), Miller et al. (2001), Dubey et al. (2003a), Dubey et al. (2003b)
Toxoplasma gondii				x		*Ej, El, Or, Ph, Pv, Tt*	Miller et al. (2001), Dubey et al. (2003b)
Fungi							
Blastomyces dermatitidis			x			*Tt*	Cates et al. (1986)
Coccidioides immitis			x			*El, Zc*	Cornell et al. (1979), Fauquier et al. (1996)
Cryptococcus gattii			x			*Lo, Pd, Pp*	Miller et al. (2002), Stephen et al. (2002)
Fusarium spp.		x				*Kb, Pv*	Frasca et al. (1996)
Sporothrix spp.		x	x		x	*Lo*	Migaki et al. (1978)

[a] Host species are abbreviated as follows: *Bm, Balaena mysticetus* (bowhead whale); *Cu, Callorhinus ursinus* (Northern fur seal); *Dl, Delphinapterus leucas* (beluga whale); *Ej, Eumetopias jubatus* (Stellar sea lion); *El, Enhydra lutris* (sea otter); *Er, Eschrichtius robustus* (gray whale); *Kb, Kogia breviceps* (pygmy sperm whale); *Lo, Lagenorhynchus obliquidens* (Pacific white-sided dolphin); *Ma, Mirounga angustirostris* (Northern elephant seal); *Or, Odobenus rosmarus* (walrus); *Pd, Phocoenoides dalli* (Dall's porpoise); *Ph, Phoca hispida* (ringed seal); *Pp, Phocoena phocoena* (harbour porpoise); *Pv, Phoca vitulina* (harbor seal); *Ta, Tursiops aduncus* (Indo-Pacific bottlenose dolphin); *Tt, Tursiops truncatus* (common bottlenose dolphin); *Zc, Zalophus californianus* (California sea lion).
[b] Raverty, S. 2014. Pers. commun. Ministry of Agriculture and Lands, Animal Health Center, Abbotsford, BC, Canada.

References: Appendix C

Arnone, R. D., and J. P. Walling. 2007. Waterborne pathogens in urban watersheds. J. Water Health 5:149–162.

Buck, J. D., L. L. Shepard, and S. Spotte. 1987. *Clostridium perfringens* as the cause of death of a captive Atlantic bottle-nosed dolphin (*Tursiops truncatus*). J. Wildl. Dis. 23:488–491.

Buck, J. D., and S. Spotte. 1986. The occurrence of potentially pathogenic vibrios in marine mammals. Mar. Mamm. Sci. 2:319–324.

Cameron, C. E., R. L. Zuerner, S. Raverty, K. A. Colegrove, S. A. Norman, D. M. Lamboum, S. J. Jeffries, and F. M. Gulland. 2008. Detection of pathogenic *Leptospira* bacteria in pinniped populations via PCR and identification of a source of transmission for zoonotic leptospirosis in the marine environment. J. Clin. Microbiol. 46:1728–1733.

Cates, M. B., L. Kaufman, J. H. Grabau, J. M. Petcher, and J. P. Schroeder. 1986. Blastomycosis in an Atlantic bottlenose dolphin. J. Am. Vet. Med. Assoc. 189:1148–1150.

Conrad, P. A., M. A. Miller, C. Kreuder, E. R. James, J. Mazet, H. Dabritz, D. A. Jessup, G. Gulland, and M. E. Grigg. 2005. Transmission of *Toxoplasma*: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. Int. J. Parasitol. 35:1155–1168.

Cornell, L. H., K. G. Osborn, J. E. Antrim, and J. G. Simpson. 1979. Coccidioidomycosis in a California sea otter (*Enhydra lutris*). J. Wildl. Dis. 15:373–378.

Danner, G. R., M. W. McGregor, R. L. Zarnke, and C. W. Olsen. 1998. Serologic evidence of influenza virus infection in a ringed seal (*Phoca hispida*) from Alaska. Mar. Mamm. Sci. 14:380–384.

Datta, K., K. H. Bartlett, R. Baer, E. Byrnes, E. Galanis, J. Heitman, L. Hoang, M. J. Leslie, L. MacDougall, S. S. Magill, M. G. Morshed, and K. A. Marr. 2009. Spread of *Cryptococcus gattii* into Pacific Northwest region of the United States. Emerg. Infect. Dis. 15:1185–1191.

Deng, M. Q., R. P. Peterson, and D. O. Cliver. 2000. First findings of *Cryptosporidium* and *Giardia* in California sea lions (*Zalophus californianus*). J. Parasitol. 86:490–494.

Dubey, J. P., D. S. Lindsay, B. M. Rosenthal, and N. J. Thomas. 2003a. Sarcocysts of an unidentified species of *Sarcocystis* in the sea otter (*Enhydra lutris*). J. Parasitol. 89:397–399.

Dubey, J. P., R. Zarnke, N. J. Thomas, S. K. Wong, W. Van Bonn, M. Briggs, J. W. Davis, R. Ewing, M. Mense, O. C. Kwok, S. Romand, and P. Thulliez. 2003b. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Vet. Parasitol. 116:275–296.

Duignan, P. J., J. T. Saliki, D. J. S. Aubin, G. Early, S. Sadove, J. A. House, K. Kovacs, and J. R. Geraci. 1995. Epizootiology of morbillivirus infection in North American harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*). J. Wildl. Dis. 31:491–501.

Fauquier, D. A., F. M. D. Gulland, J. G. Trupkiewicz, T. R. Spraker, and L. J. Lowenstine. 1996. Coccidioidomycosis in free-living California sea lions (*Zalophus californianus*) in central California. J. Wildl. Dis. 32:707–710.

Frasca, Jr., S., J. L. Dunn, J. C. Cooke, and J. D. Buck. 1996. Mycotic dermatitis in an Atlantic white-sided dolphin, a pygmy sperm whale, and two harbor seals. J. Am. Vet. Med. Assoc. 208:727–729.

Fravel, V., and R. H. Evans. 2008. Staphylococcal pyelonephritis and cystitis in a California sea lion (*Zalophus californianus*). J. Mar. Anim. Their Ecol. 1:11–13.

Fujioka, R. S., S. B. Greco, M. B. Cates, and J. P. Schroeder. 1988. *Vibrio damsela* from wounds in bottlenose dolphins *Tursiops truncatus*. Dis. Aquat. Organ. 4:1–8.

Gilmartin, W. G., P. M. Vainik, and V. M. Neill. 1979. Salmonellae in feral pinnipeds off the southern California coast. J. Wildl. Dis. 15:511–514.

Godfroid, J., A. Cloeckaert, J. P. Liautard, S. Kohler, D. Fretin, K. Walravens, B. Garin-Bastuji, and J. J. Letesson. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet. Res. 36:313–326.

Hughes-Hanks, J. M., L. G. Rickard, C. Panuska, J. R. Saucier, T. M. O'Hara, L. Dehn, and R. M. Rolland. 2005. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in five marine mammal species. J. Parasitol. 91:1225–1228.

Imai, D., S. Jang, M. Miller, and P. A. Conrad. 2009. Characterization of beta-hemolytic streptococci isolated from southern sea otters (*Enhydra lutris nereis*) stranded along the California coast. Vet. Microbiol. 136:378–381.

Jagger, T., S. Keane, and S. Robertson. 2000. Plesiomonas shigelloides—an uncommon cause of diarrhoea in cats? Vet. Rec. 146:296.

Jang, S., L. Wheeler, R. B. Carey, B. Jensen, C. M. Crandall, K. N. Schrader, D. Jessup, K. Colegrove, and F. M. D. Gulland. 2010. Pleuritis and suppurative pneumonia associated with a hypermucoviscosity phenotype of *Klebsiella pneumoniae* in California sea lions (*Zalophus californianus*). Vet. Microbiol. 141:174–177.

Kersh, G. J., D. M. Lambourn, J. S. Self, A. M. Akmajian, J. B. Stanton, T. V. Baszler, S. A. Raverty, and R. F. Massung. 2010. *Coxiella burnetii* infection of a steller sea lion (*Eumetopias jubatus*) found in Washington State. J. Clin. Microbiol. 48:3428–3431.

Lambourn, D. M., S. J. Jeffries, M. M. Garner, D. R. Ewalt, S. A. Raverty, J. K. Gaydos, and J. C. Rhyan. 2006. Evidence of *Brucella* sp. infection in Pacific harbor seals (*Phoca vitulina richardsii*) 1993–2004 from Washington. Poster presented at the 37th Annual International Association for Aquatic Animal Medicine Conference, 6–10 May 2006, Nassau, Bahamas.

Lapointe, J. M., P. J. Duignan, A. E. Marsh, F. M. Gulland, B. C. Barr, D. K. Naydan, D. P. King, C. A. Farman, K. A. Huntingdon, and L. J. Lowenstine. 1998. Meningoencephalitis due to a *Sarcocystis neurona*-like protozoan in Pacific harbor seals (*Phoca vitulina richardsi*). J. Parasitol. 84:1184–1189.

Lapointe, J. M., F. M. Gulland, D. M. Haines, B. C. Barr, and P. J. Duignan. 1999. Placentitis due to *Coxiella burnetii* in a Pacific harbor seal (*Phoca vitulina richardsi*). J. Vet. Diagn. Invest. 11:541–543.

Lloyd-Smith, J. O., D. J. Greig, S. Hietala, G. S. Ghneim, L. Palmer, J. St Leger, B. T. Grenfell, and F. M. Gulland. 2007. Cyclical changes in seroprevalence of leptospirosis in California sea lions: Endemic and epidemic disease in one host species? BMC Infect. Dis. 7:1–11.

MacDougall, L., S. E. Kidd, E. Galanis, S. Mak, M. J. Leslie, P. R. Cieslak, J. W. Kronstad, M. G. Morshed, and K. H. Bartlett. 2007. Spread of *Cryptococcus gattii* in British Columbia, Canada, and detection in the Pacific Northwest, U.S.A. Emerg. Infect. Dis. 13:42–50.

Migaki, G., R. L. Font, W. Kaplan, and E. D. Asper. 1978. Sporotrichosis in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). Am. J. Vet. Res. 39:1916–1919.

Miller, M. A., I. A. Gardner, C. Kreuder, D. M. Paradies, K. R. Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham, and P. A. Conrad. 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). Int. J. Parasitol. 32:997–1006.

Miller, M. A., K. Sverlow, P. R. Crosbie, B. C. Barr, L. J. Lowenstine, F. M. Gulland, A. Packham, and P. A. Conrad. 2001. Isolation and characterization of two parasitic protozoa from a Pacific harbor seal (*Phoca vitulina richardsi*) with meningoencephalomyelitis. J. Parasitol. 87:816–822.

Palmer, C. J., J. P. Schroeder, R. S. Fujioka, and J. T. Douglas. 1991. *Staphylococcus aureus* infection in newly captured Pacific bottlenose dolphins (*Tursiops truncatus gilli*). J. Zoo Wildl. Med. 22:330–338.

Raverty, S. A., J. K. Gaydos, K. Nielsen, O. Nielsen, P. S. Ross, D. M. Lambourn, and S. J. Jeffries. 2005. An overview of marine mammal diagnoses in the Pacific Northwest from 1999 to 2004. Poster presented at the 36th Annual International Association for Aquatic Animal Medicine Conference, 14–19 May 2005, Seward, AK.

Schroeder, J. P., J. G. Wallace, M. B. Cates, S. B. Greco, and P. W. B. Moore. 1985. An infection by *Vibrio alginolyticus* in an Atlantic bottlenose dolphin housed in an open ocean pen. J. Wildl. Dis. 21:437–438.

Siebert, U., G. Muller, G. Desportes, R. Weiss, K. Hansen, and W. Baumgartner. 2002. Pyogranulomatous myocarditis due to *Staphylococcus aureus* septicaemia in two harbour porpoises (*Phocoena phocoena*). Vet. Rec. 150:273–277.

Smith, A. W., D. E. Skilling, N. Cherry, J. H. Mead, and D. O. Matson. 1998. Calicivirus emergence from ocean reservoirs: Zoonotic and interspecies movements. Emerg. Infect. Dis. 4:13–20.

Steiger, G. H., J. Calambokidis, J. C. Cubbage, D. E. Skilling, A. W. Smith, and D. H. Gribble. 1989. Mortality of harbor seal pups at different sites in the inland waters of Washington. J. Wildl. Dis. 25:319–328.

Stephen, C., S. Lester, W. Black, M. Fyfe, and S. Raverty. 2002. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. Can. Vet. J. 43:792–794.

Stoddard, R. A., E. R. Atwill, F. M. D. Gulland, M. A. Miller, H. A. Dabritz, D. M. Paradies, K. R. Worcester, S. Jang, J. Lawrence, B. A. Byrne, and P. A. Conrad. 2008. Risk factors for infection with pathogenic and antimicrobial-resistant fecal bacteria in northern elephant seals in California. Public Health Rep. 123:360–370.

Thornton, S.M., S. Nolan, and F. M. D. Gulland. 1998. Bacterial isolates from California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seals (*Mirounga angustirostris)* admitted to a rehabilitation center along the central California coast, 1994–1995. J. Zoo Wildl. Med. 29:171–176.

Upton, A., J. A. Fraser, S. E. Kidd, C. Bretz, K. H. Bartlett, J. Heitman, and K. A. Marr. 2007. First contemporary case of human infection with *Cryptococcus gattii* in Puget Sound: Evidence for spread of the Vancouver Island outbreak. J. Clin. Microbiol. 45:3086–3088.

Van Bressem, M. F., J. A. Raga, G. Di Guardo, P. D. Jepson, P. J. Duignan, U. Siebert, T. Barrett, M. C. Santos, I. B. Moreno, S. Siciliano, A. Aguilar, and K. Van Waerebeek. 2009. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Dis. Aquat. Organ. 86:143–157.

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NOAA Technical Memorandum NMFS-NWFSC-

134 Leonard, J. 2016. Washington and Oregon charter vessel survey: Methodology and results. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-134, 22 p. doi:10.7289/V5/TM-NWFSC-134.

133 Duffield, D., J. K. Gaydos, S. Raverty, K. Wilkinson, B. Norberg, L. Barre, M. B. Hanson, P. Foreman, A. Traxler, D. Lambourn, J. Huggins, J. Calambokidis, T. McKlveen, S. Dennison, and H. Brubaker. 2016. Wild animal mortality investigation: Southern Resident killer whale L-112 final report. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-133, 122 p. doi:10.7289/V5/TM-NWFSC-133.

132 Anderson, L. E., and M. Plummer. 2016. Puget Sound recreational shellfishing survey: Methodology and results. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-132, 38 p. doi:10.7289/V5/TM-NWFSC-132.

131 Waples, R. S. 2016. Small investments with big payoffs: A decade of the Internal Grants Program at the Northwest Fisheries Science Center. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-131, 80 p. doi:10.7289/V5/TM-NWFSC-131.

130 Puget Sound Recovery Implementation Technical Team. 2015. Puget Sound Chinook salmon recovery: A framework for the development of monitoring and adaptive management plans. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-130, 146 p. NTIS number PB2016-100691. doi:10.7289/V5/TM-NWFSC-130.

129 Hard, J. J., J. M. Myers, E. J. Connor, R. A. Hayman, R. G. Kope, G. Lucchetti, A. R. Marshall, G. R. Pess, and B. E. Thompson. 2015. Viability criteria for steelhead within the Puget Sound distinct population segment. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-129, 332 p. NTIS number PB2015-105188. doi:10.7289/V5/TM-NWFSC-129.

128 Myers, J. M., J. J. Hard, E. J. Connor, R. A. Hayman, R. G. Kope, G. Lucchetti, A. R. Marshall, G. R. Pess, and B. E. Thompson. 2015. Identifying historical populations of steelhead within the Puget Sound distinct population segment. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-128, 155 p. NTIS number PB2015-103741. doi:10.7289/V5/TM-NWFSC-128.

127 Roni, P., G. R. Pess, T. J. Beechie, and K. M. Hanson. 2014. Fish–habitat relationships and the effectiveness of habitat restoration. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-127, 154 p. NTIS number PB2014-108836.

126 Russell, S., and M. S. Ruff. 2014. The U.S. whale watching industry of Greater Puget Sound: A description and baseline analysis. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-126, 171 p. NTIS number PB2014-105939.

NOAA Technical Memorandums NMFS-NWFSC are available at the Northwest Fisheries Science Center website, https://www.nwfsc.noaa.gov/index.cfm.

Spring 2012

Water Quality

How Toxic Runoff Affects Pacific Salmon & Steelhead

Development in the Pacific Northwest has transformed how and when water moves through the landscape, and how species respond to the physical, chemical, and biological changes in their environment – in short, how our watersheds function. As our human population grows, so do the number of motor vehicles on the roads and types of pollutants released across the landscape. Deposits of pollutants end up on roadways and other surfaces, and are transported to our waterways via runoff. Contaminated runoff poses significant threats to freshwater, estuarine, and marine species, including the Pacific Northwest's salmon and steelhead runs.

What Science Tells Us About Salmon & Polluted Runoff

Pavement is a prominent feature of the landscape. Roadways and parking lots are impervious and accumulate a mixture of contaminants, including metals (copper, nickel, zinc, cadmium, etc.); petroleum-derived compounds from oil, grease, and vehicle exhaust; and detergents, among others. During rainfall events, stormwater collects these contaminants and transports them to our rivers, lakes, and estuaries. In addition, agricultural practices and landscape maintenance that use pesticides, such as insecticides, herbicides, and fungicides, can also contaminate runoff and compromise the health of watersheds.

When toxics enter our waterways via stormwater runoff, they can cause a variety of adverse effects to aquatic species. In addition to directly impacting salmon and steelhead, toxics can harm or kill the aquatic insects that salmon eat. Pollution risks vary depending on the particular chemical, the amount transported in stormwater, and environmental persistence.

Recent research has shown that common contaminants can impair salmon health in a variety of ways. For example, certain metals and pesticides are toxic to the salmon nervous system, thereby disrupting feeding and predator avoidance. Pesticides and petroleum-derived compounds suppress the immune system, rendering salmon more vulnerable to pathogens that cause lethal diseases. Petrolem-derived compounds are also known to depress growth rate of juvenile salmon, which can affect their survival. Other compounds target the developing cardiovascular system, causing heart failure or permanent heart defects.

Dissolved copper is a particularly pervasive contaminant that threatens salmon and steelhead survival. Copper is used for many industrial, commercial, and residential purposes. These include use in roofing materials, treated wood, and pesticides. It is also a by-product found in the exhaust and brake pads of vehicles. Copper, like many other metals, is toxic to the sensory systems of fish. Dissolved copper specifically impairs salmon and steelhead's ability to detect odors. This sense guides their response to environmental cues and impairment of smell interferes with certain behaviors. Copper can impede predator detection and avoidance, social interaction, prey detection, orientation, and homing, thereby affecting their survival, distribution, and reproductive success.

Copper is just one example of a contaminant in stormwater. In the natural world, fish are exposed to a mixture of chemicals originating from a variety of sources. Although the specific

NOAA FISHERIES SERVICE

What is stormwater runoff?

Stormwater is rain or snow melt that originates during precipitation events. Stormwater that does not soak into the ground becomes runoff.

How do pollutants enter our waterways?

Pavement is part of our landscape. Roadways and parking lots accumulate a mixture of contaminants, such as metals and petroleum-related compounds. During precipitation events, stormwater runoff transports the pollutants to our rivers, lakes, and estuaries. Agricultural chemicals, such as pesticides, can also seep into waterways where pollutants are transported downstream.

responses of salmon and steelhead to the mixtures present in stormwater are difficult to predict, they could include many of the effects described above. Research at the Northwest Fisheries Science Center has shown that typical stormwater mixtures affect the survival and development of salmon and steelhead eggs.

Best Practices for Cleaning-Up Toxic Runoff

Using its authority under the Endangered Species Act, NOAA Fisheries has worked with local governments and Federal partners, including the Federal Highway Administration, Federal Transit Authority, and U.S. Department of Housing and Urban Development to improve water quality in salmon habitats by reducing toxic runoff. Some best practices include:

Infiltration: The primary means of treating polluted runoff is infiltration. There are many creative ways to infiltrate polluted runoff into the subsurface where it can be cleaned using natural materials like compost-amended soils. Local developments also are incorporating environmentally sound development techniques, known as "low-impact development," into their buildings and roads. These techniques include things such as adding green roofs, pervious pavement, rain gardens, compost-amended soils, wetlands, and vegetative filter strips along driveways and walkways.

High Efficiency Sweepers: Major cities like Seattle and Portland are using high efficiency sweepers to collect and dispose of runoff pollutants before they are transported into streams and rivers. This approach can remove up to 90% of the pollutants (e.g., copper and zinc) from roadways.

Reduce Pollution at the Source: Source control is the most effective tool for reducing or eliminating toxics in stormwater runoff. For example, Washington State recently passed legislation that will phase out the use of copper and other metals in vehicle brake pads, and some jurisdictions have banned the use of architectural copper (downspouts, etc.). Many local governments have implemented public education campaigns to reduce the use of toxic lawn care products. With the support of over $50 million in Federal grants, Seattle and Portland are piloting the use of electric vehicles and charging stations. This change will reduce the amounts and sources of oil, gas, and metals on our roadways.

For More Information

If you would like to learn more about how polluted runoff affects salmon and steelhead, or if you would like to incorporate best management practices into your projects, please consider the following sources:

NOAA's Coastal Storms Program
- http://coastalstorms.noaa.gov/stormwater/

NOAA Fisheries' Northwest Fisheries Science Center Research
- www.nwfsc.noaa.gov/research/divisions/ec/ecotox/fishneurobiology/index.cfm
- www.nwr.noaa.gov/Salmon-Habitat/upload/toxics-rsrch.pdf

Washington Stormwater Center for Low Impact Development Research & Resources
- www.wastormwatercenter.org/low-impact/

Western Washington State Stormwater Manual
- www.ecy.wa.gov/programs/wq/stormwater/manual.html

City of Portland Stormwater Management Manual
- www.portlandonline.com/bes/index.cfm?c=47952

Environmental Protection Agency for Green Infrastructure Tools
- http://water.epa.gov/infrastructure/greeninfrastructure/

Idaho Department of Environmental Quality
- www.deq.idaho.gov/water-quality/wastewater/stormwater.aspx

Washington State Department of Transportation Stormwater Research
- www.wsdot.wa.gov/Environment/WaterQuality/Research/

Guidance Document for Preparation of the NPDES Stormwater Pollution Control Plan
- www.deq.state.or.us/wq/stormwater/docs/nwr/swpcpguide.pdf

Be the change you want to see!

Take steps to ensure runoff is not polluted because clean water is a matter of health and safety for both fish and people.

BEFORE THE UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY

Petition for Rulemaking to Implement	)
Reasonable and Prudent Alternatives in	)
Biological Opinions from the U.S. Fish and	)
Wildlife Service and National Marine	)
Fisheries Service for Toxic Water Quality	)
Criteria in Idaho Water Quality Standards	)

I. Introduction

For the reasons detailed below, Northwest Environmental Advocates ("NWEA") hereby petitions the U.S. Environmental Protection Agency ("EPA") to promulgate rules implementing reasonable and prudent alternatives ("RPAs") included in the 2014 National Marine Fisheries Service's ("NMFS") and 2015 U.S. Fish and Wildlife Service's ("FWS") and Biological Opinions for the Idaho Water Quality Standards for Numeric Water Quality Criteria for Toxic Pollutants (the "Proposed Action").[1] These RPAs require EPA to promulgate aquatic life criteria for Idaho waters for chronic arsenic, acute and chronic cyanide, chronic lead, acute and chronic nickel, acute and chronic zinc, and to remove the low hardness floor. As indicated in the Biological Opinions, implementation of these RPAs is needed to avoid jeopardy to numerous species listed as threatened or endangered under the federal Endangered Species Act ("ESA"), including Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon,

[1] *See generally*, National Marine Fisheries Service, Final Endangered Species Act Section 7 Formal Consultation and Magnuson-Stevens Fishery Conservation and Management Act Essential Fish Habitat Consultation for Water Quality Toxics Standards for Idaho, NMFS No. 2000-1484 (May 7, 2014), *available at* https://www.northwestenvironmentaladvocates.org/blog/wp-content/uploads/2014/07/2014_05_07-NMFS-BiOp-Idaho-Toxics.pdf (last visited 7 Feb. 2023) (hereafter, "NMFS Biological Opinion"); Fish and Wildlife Service, Biological Opinion for the Idaho Water Quality Standards for Numeric Water Quality Criteria for Toxic Pollutants, OlEIFW00-2014-F-0233 (June 15, 2015), *available at* https://northwestenvironmentaladvocates.org/wpdm-package/fws-idaho-biop-toxics/ (hereafter, "FWS Biological Opinion").

Snake River Basin steelhead, Snake River physa, Bliss Rapids snail, Banbury Springs lanx, Bruneau hot springsnail, bull trout, and Kootenai River white sturgeon. Yet EPA has not implemented the RPAs for these criteria. Absent implementation of these RPAs, EPA is likely causing or contributing to the take of these listed species in violation of the ESA.

Moreover, as demonstrated in the following chart, the dates for completion of the RPAs set out in the Biological Opinions have all passed, as have the dates for EPA to initiate ESA consultation on the criteria adopted pursuant to the RPAs. Because EPA has not even completed these RPAs, EPA has not, and could not have met, the deadlines to initiate consultation ESA consultation on the RPAs.

Date RPA published	**Aquatic Life Criteria or Action Required by the RPA**	**Date for RPA Completion**	**Date for EPA initiation of ESA consultation for RPA**
2014 NMFS 2015 FWS	Removal of hardness floor	May 7, 2017	–
2014 NMFS 2015 FWS	Chronic arsenic	May 7, 2021	May 7, 2020 December 23, 2020
2015 FWS	Acute and chronic cyanide	May 7, 2021	December 23, 2020
2015 FWS	Acute and chronic nickel	May 7, 2022	December 23, 2021
2015 FWS	Acute and chronic zinc	May 7, 2022	December 23, 2021
2015 FWS	Chronic lead	May 7, 2023	December 23, 2022

This petition is brought pursuant to the Administrative Procedure Act ("APA"), which requires that "[e]ach agency shall give an interested person the right to petition for the issuance, amendment, or repeal of a rule."[2] The APA further imposes an obligation on EPA to timely respond to this petition, by requiring that "[w]ith due regard for the convenience and necessity of the parties or their representatives and within a reasonable time, each agency shall proceed to conclude a

[2] 5 U.S.C. § 553(e).

matter presented to it."[3] Timely notice includes not only affirmative action but also any decision to deny this petition, in whole or in part.[4]

II. Legal Background

Congress adopted amendments to the Clean Water Act ("CWA") in 1972 in order "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters."[5] The CWA establishes an "interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife[.]"[6]

To meet these goals, the CWA requires states to develop water quality standards that establish, and then protect, the desired conditions of each waterway within the state's regulatory jurisdiction.[7] Among other things, water quality standards include numeric and narrative criteria specifying the water quality conditions—such as maximum concentrations of toxic pollutants—that are necessary to protect the designated uses.[8] Water quality standards must be sufficient to "protect the public health or welfare, enhance the quality of water, and serve the purposes of [the CWA]."[9]

States must review their water quality standards at least every three years and submit all new and revised water quality standards to EPA for review and approval.[10] A state-developed water quality standard does not become effective until EPA approves it.[11] If EPA approves a new or revised standard, it must notify the state within 60 days of the state's submission of the

[3] *Id*. § 555(b).
[4] *Id*. § 555(e).
[5] 33 U.S.C. § 1251(a).
[6] *Id*. § 1251(a)(2).
[7] *Id*. § 1313(a).
[8] *Id*. §§ 1313(c)(2); 1313(d)(4)(B); 40 C.F.R. Part 131, Subpart B.
[9] 33 U.S.C. § 1313(c)(2)(A).
[10] *Id*. §§ 1313(c)(1), (3).
[11] *Id*. § 1313(c)(3); 40 C.F.R. § 131.21(c).

standard.[12] EPA must then review and approve or disapprove any revised or new standards for consistency with the federal CWA.[13]

If EPA determines that a standard is not consistent with the requirements of the CWA, within 90 days of the state's submission, EPA must notify the state of EPA's intent to disapprove the standard and specify changes to the standard that are necessary to comply with the CWA.[14] If the state does not cure the problems with the standard within a second 90-day period, EPA must "promptly" promulgate a substitute standard.[15] EPA must also establish new or revised water quality standards whenever the agency determines that new or revised standards are necessary to meet the requirements of the CWA.[16]

The CWA's requirements often intersect with those of the federal Endangered Species Act ("ESA") because many species listed as threatened or endangered pursuant to the ESA are found in or depend on water for their survival. The ESA's purpose is to "provide a program for the conservation of . . . endangered species and threatened species" and to "provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved[.]"[17] One overarching requirement of the ESA is that all federal departments and agencies must "seek to conserve" threatened and endangered species.[18] The terms "conserve" and "conservation" mean "to use and the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures provided

[12] 33 U.S.C. § 1313(c)(3).
[13] *Id*. § 1313(c)(2).
[14] *Id*. § 1313(c)(3).
[15] *Id*.; *id*. § 1313(c)(4)(A).
[16] *Id*. § 1313(c)(4)(B).
[17] 16 U.S.C. § 1531(b).
[18] *Id*. § 1531(c)(1).

pursuant to [the ESA] are no longer necessary."[19] In addition, all federal agencies must, in consultation with and with assistance from the Secretaries of Interior and Commerce—the Secretaries vested with responsibility for administering the ESA—"utilize their authorities in furtherance of the purposes of [the ESA] by carrying out programs for the conservation of" ESA-listed species.[20]

The ESA requires the Secretary of Interior or Commerce to list species that the Secretary believes may become extinct in the near future as being either "threatened" or "endangered."[21] A species is "endangered" if it "is in danger of extinction throughout all or a significant portion of its range."[22] A species is "threatened" if it "is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range."[23]

Under ESA Section 7, all federal agencies must ensure that "any action authorized, funded, or carried out by such agency . . . is not likely to jeopardize the continued existence of any endangered species or threatened species or result in the destruction or adverse modification of [critical] habitat of such species[.]"[24] The ESA's implementing regulations define "jeopardy" to an endangered or threatened species as "an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed

[19] *Id*. § 1532(3).
[20] Id. § 1536(a)(1).
[21] *Id*. § 1533.
[22] *Id*. § 1532(6).
[23] *Id*. § 1532(20).
[24] *Id*. § 1536(a)(2). The ESA defines critical habitat as two specific categories. First, "the specific areas within the geographical area occupied by the species, at the time it is listed in accordance with the provisions of section 1533 of this title, on which are found those physical or biological features (I) essential to the conservation of the species and (II) which may require special management considerations or protection." *Id*. § 1532(5)(A)(i). Second, critical habitat means "specific areas outside the geographical area occupied by the species at the time it is listed in accordance with the provisions of section 1533 of this title, upon a determination by the Secretary that such areas are essential for the conservation of the species." *Id.* § 1532(5)(A)(ii).

species."[25] Agencies must also ensure that agency actions are not likely to "result in the destruction or adverse modification of [critical] habitat."[26] This is a separate determination from whether the action will jeopardize the continued existence of threatened or endangered species. EPA's approval of a state's proposed water quality standard is an agency action subject to section 7.[27]

Whenever a federal agency determines that a proposed action may affect one or more ESA-listed species it must consult with NMFS and/or the FWS (together the "Services"), depending on the species.[28] The "may affect" threshold that triggers ESA section 7 consultation is low: "any possible effect, whether beneficial, benign, adverse, or of an undetermined character, triggers the formal consultation requirement."[29] A federal agency proposing an action that "may affect" a listed species must prepare and provide to the relevant Service a "biological assessment" ("BA") of the effects of the proposed action.[30] For those actions that may affect a listed species, the Service must review all information provided by the action agency, as well as any other relevant information, to determine whether the proposed action is likely to jeopardize a listed species or destroy or adversely modify its designated critical habitat.[31] This determination is set forth in a "biological opinion" from one or both of the Services.[32] If the Service concludes that the proposed action is likely to jeopardize a listed species or destroy or adversely modify its critical habitat, it must

[25] 50 C.F.R. § 402.02.
[26] *Id.*; *see also id.* § 402.14(g)(4).
[27] *See* Memorandum of Agreement Between the Environmental Protection Agency, Fish and Wildlife Service and National Marine Fisheries Service Regarding Enhanced Coordination Under the Clean Water Act and Endangered Species Act, 66 Fed. Reg. 11,202, 11,214 (Feb. 22, 2001) ("Section 7 consultation is required if EPA determines that its approval of any of the [state or tribal water quality standards] may affect listed species or designated critical habitat.").
[28] 50 C.F.R. § 402.14(a).
[29] *W. Watersheds Project v. Kraayenbrink*, 632 F.3d 472, 496 (9th Cir. 2011) (citing 51 Fed. Reg. 19,926, 19,949 (June 3, 1986)).
[30] 16 U.S.C. §§ 1536(a)(2), (c); 50 C.F.R. § 402.14(a). These may also be termed "biological evaluations."
[31] 50 C.F.R. § 402.14(g)–(h).
[32] *Id*. § 402.14(h); 16 U.S.C. § 1536(b)(3)(A).

identify and describe any reasonable and prudent alternatives ("RPAs") to the proposed action that it believes would avoid jeopardy and adverse modification.[33] If the agency believes there is no RPA, the biological opinion must so state.[34]

Implementation of RPAs is technically optional, but only to the extent that the action agency can choose to either implement the RPAs or assume the risk of taking an action which may cause illegal take of ESA-listed species.[35] In *Bennett v. Spear*, the U.S. Supreme Court clarified that RPAs in a biological opinion are essentially a set of "terms and conditions" that an action agency must follow in order for the biological opinion's incidental take statement[36] to be applicable to the action.[37] If the agency chooses to not implement the RPAs, "it does so at its own peril (and that of its employees), for 'any person' who knowingly 'takes' an endangered or threatened species is subject to substantial civil and criminal penalties[.]"[38]

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[33] 16 U.S.C. § 1536(b)(3)(A). Jeopardy findings have become increasingly rare. A 2015 analysis of seven years of FWS consultation found that out of 81,461 informal and 6,829 formal consultations, only two resulted in jeopardy determinations. Jacob W. Malcom and Ya-Wei Li, *Data contradict common perceptions about a controversial provision of the US Endangered Species Act*, 112 Proceedings of the National Academy of Sciences 15844, 15845 (Dec. 2015) (hereinafter "Malcom and Li").
[34] 50 C.F.R. § 402.14(h)(2).
[35] When RPAs are not feasible, the action cannot move forward absent an exemption by a special committee. Malcom and Li, *supra* n.33 at 15845; *see also*, 16 U.S.C. § 1536(g)(3)(A).
[36] Where one of the Services concludes in a biological opinion that an action will not jeopardize the continued existence of a listed species but may result in incidental takings of listed species, the Service must include a written "incidental take statement" in the biological opinion authorizing such takings. *See ONRC v. Allen*, 476 F.3d 1031, 1034 (9th Cir. 2007); *see also* 16 U.S.C. §§ 1536(b)(4), (o).
[37] *Bennett v. Spear*, 520 U.S. 154, 169–70 (1997).
[38] *Id*. at 170.

III. EPA has Failed to Implement RPAs Designed to Protect Idaho's ESA-Listed Species from Jeopardy.

In 2013, NWEA brought suit against NMFS, FWS, and EPA for failing to carry out their mandatory statutory duties under the CWA and ESA.[39] Among other things, NWEA alleged that the Services had failed to complete Section 7 consultation for EPA's action of approving Idaho's 1994 and 1997 toxic criteria.[40] Despite having prepared draft biological opinions finding jeopardy, the Services had still—more than 10 years later—not produced final biological opinions.[41] The suit resulted in a court-enforceable settlement that required the Services to complete their much-delayed biological opinions related to EPA's approval of Idaho's toxic water quality standards.[42]

As a result of NWEA's lawsuit—and after over 17 years of delay—in 2014 and 2015, respectively, NMFS and FWS finally fulfilled their ESA Section 7 responsibilities by releasing final Biological Opinions that concluded EPA's approval of certain Idaho water quality standards jeopardizes certain listed species and destruction or adverse modification of their critical habitats.[43] Specifically, NMFS made the following jeopardy determinations:

- Potential effects of using **25 mg/L hardness floor** in calculating metals discharge limits will rise to the level of jeopardizing the Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and will result in the adverse modification of designated critical habitat for these species.[44]

[39] *See* Stipulated Order of Dismissal of All Claims Against Defendants National Marine Fisheries Service and U.S. Fish and Wildlife Service, *Northwest Environmental Advocates v. The National Marine Fisheries Service,* No. 1:13-cv-00263-EJL (2015), ECF No. 37 at 1–3.
[40] *Id*. at 2–3.
[41] *Id*. at 2.
[42] *Id*. at 3–4.
[43] The FWS and NMFS Biological Opinions were conducted for purposes of EPA's approval of certain Idaho water quality criteria (the "Proposed Action"). The history of these criteria, and EPA's review and consultation over them, is complex, and set forth in the FWS and NMFS Biological Opinions. *See* NMFS Biological Opinion at 1–4; FWS Biological Opinion at 1–2. The specific criteria over which EPA was consulting on for purposes of the Biological Opinions are set forth Table 1.3.1 of NMFS's Biological Opinion. *See* NMFS Biological Opinion at 6–8; *see also* FWS Biological Opinion at Table 1, pp. 8–10.
[44] NMFS Biological Opinion at 274–75.

- Potential effects of the proposed **chronic arsenic criterion** of 150 µg/L would jeopardize the Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and is likely to result in the adverse modification of designated critical habitat for these species.[45]

- Potential effects of the proposed **acute and chronic copper criteria** are likely to jeopardize the Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and are likely to result in the adverse modification of designated critical habitat for these species.[46]

- Potential effects of the proposed **chronic cyanide criterion** of 5.2 µg/L will jeopardize the Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and result in the adverse modification of designated critical habitat for these species.[47]

- Potential effects of the proposed **chronic mercury criterion** of .012 µg/L will jeopardize the Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and will adversely modify designated critical habitat for these species.[48]

- Potential effects of the proposed **chronic selenium criterion** of 5 µg/L will jeopardize Snake River spring/summer chinook salmon, Snake River fall chinook salmon, Snake River sockeye salmon, and Snake River Basin steelhead, and will adversely modify designated critical habitat for these species.[49]

Given these jeopardy determinations, NMFS's Biological Opinion included a series of final RPAs that, if implemented, NMFS believed would avoid jeopardizing the continued existence of the listed species and avoid the destruction or adverse modification of critical habitat.[50] The final outstanding RPAs and their status are as follows:

[45] *Id*. at 6, 275.
[46] *Id*. at 276.
[47] *Id*. at 6, 276.
[48] *Id*.
[49] *Id*. at 6, 277.
[50] *Id*. at 281; *see also*, 16 U.S.C. §1536(b)(3)(A).

- Removal of the **low-end hardness floor** by May 7, 2017.[51] *EPA failed to implement this RPA by the deadline and to date has not promulgated the RPA.*[52] Furthermore, NMFS strongly encouraged IDEQ to consider removing the hardness floor in a comment on IDEQ's 2020 triennial review.[53] IDEQ expressly rejected the suggestion.[54]

- Ensure, either through EPA promulgation of a criterion or EPA approval of a state-promulgated criterion, that a new **chronic criterion for arsenic** is in effect in Idaho by May 7, 2021 and is consistent with the discussion and analysis in the NMFS Biological Opinion.[55] Initiation of consultation on a new criterion was required by May 7, 2020. *EPA has failed to implement this RPA by the deadline and to date has not promulgated a criterion consistent with the RPA*.[56]

- Ensure, either through EPA promulgation of a criterion or EPA approval of a state-promulgated criterion, that a new **chronic criterion for mercury** is in effect in Idaho by May 7, 2021 and that the criterion is consistent with the discussion and analysis in the NMFS Biological Opinion.[57] Initiation of consultation on a new criterion was required by May 7, 2020. On October 4, 2022, EPA and NWEA entered into a stipulated order that extended the deadline for implementing the mercury RPA until April 4, 2024 (18 months after the entry of the stipulated order).[58]

As noted, FWS also made a number of jeopardy determinations in its Biological Opinion.

Specifically, FWS determined that:

- The proposed **chronic arsenic criterion** of 150 µg/L level likely is likely to jeopardize the Snake River physa, Bliss Rapids snail, Banbury Springs lanx, Bruneau hot springsnail, bull trout, and Kootenai River white sturgeon, and is likely to adversely modify designated critical habitat for these species.[59]

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[51] NMFS Biological Opinion at 281.
[52] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.c.i. https://adminrules.idaho.gov/rules/current/58/580102.pdf. (last visited 21 Feb. 2023).
[53] Idaho DEQ, *2020 Triennial Review of Idaho Water Quality Standards*, 10 (2020), https://www2.deq.idaho.gov/admin/LEIA/api/document/download/15165.
[54] *Id*.
[55] NMFS Biological Opinion at 282.
[56] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.a.
[57] NMFS Biological Opinion at 284.
[58] Stipulated Order on Remedy, *Northwest Environmental Advocates v. The National Marine Fisheries Service,* No. 1:13-cv-00263-DCN (2022), ECF No. 119. This stipulated order on remedy requires EPA to issue draft mercury criteria by March 30, 2024; determine whether ESA consultation is required within 9 months of that publication; and sign a final criteria rule within 8 months of concluding the ESA consultation or, if EPA determines no consultation is required, finalize the rule within 8 months of that determination. *Id*. at ¶ I.1–4.
[59] FWS Biological Opinion at 258.

- The proposed **acute and chronic copper criteria** are likely to jeopardize the Snake River physa, Bliss Rapids snail, Banbury Springs lanx, Bruneau hot springsnail, bull trout, and Kootenai River white sturgeon, and is likely to adversely modify designated critical habitat for the fish species.[60]

- The proposed **acute and chronic cyanide criteria** of 22 µg/L and 5.2 µg/L, respectively, are likely to jeopardize bull trout and Kootenai River white sturgeon, and are likely to adversely modify designated critical habitat for these species.[61]

- The proposed **chronic lead criterion** is likely to jeopardize the Banbury Springs lanx.[62]

- The proposed **chronic mercury criterion** of 0.012 µg/L is likely to jeopardize bull trout and Kootenai River white sturgeon, and is likely to adversely modify designated critical habitat for these species.[63]

- The proposed **chronic selenium criterion** of 5 µg/L is likely to jeopardize bull trout and Kootenai River white sturgeon and is likely to adversely modify designated critical habitat for these species.[64]

- The proposed **acute and chronic zinc criteria** are likely to jeopardize bull trout and Kootenai River white sturgeon, and are likely to adversely modify designated critical habitat for these species.[65]

- The proposed **acute and chronic nickel criteria** are likely to jeopardize the Snake River physa, Bliss Rapids snail, Banbury Springs lanx, and Bruneau hot springsnail.[66]

Like NMFS's Biological Opinion, FWS's Biological Opinion included a series of final RPAs that, if taken, FWS believed would avoid jeopardizing the continued existence of the listed species and avoid the destruction or adverse modification of critical habitat.[67] The final outstanding RPAs and their status are:

- Ensure, either through EPA promulgation of criteria or EPA approval of a state-promulgated criteria, that **new acute and chronic criteria for cyanide** are in effect in

[60] *Id*.
[61] *Id*.
[62] *Id*.
[63] *Id*.
[64] *Id*.
[65] *Id*.
[66] *Id*. at 259.
[67] *Id*. at 267–68; *see also*, 16 U.S.C. § 1536(b)(3)(A).

Idaho by May 7, 2021 and are consistent with the discussion and analysis in the FWS Biological Opinion.[68] Initiation of consultation on new criteria was required by December 23, 2020. *EPA has failed to implement this RPA by the deadline and to date has not promulgated the RPA*.[69]

- Ensure, either through EPA promulgation of a criterion or EPA approval of a state-promulgated criterion, that **a new chronic criterion for lead** is in effect in Idaho by May 7, 2023 and is consistent with the discussion and analysis in the FWS Biological Opinion.[70] Initiation of consultation on a new criterion was required by December 23, 2022. *EPA has failed to implement this RPA by the deadline and to date has not promulgated the RPA*.[71]

- Ensure, either through EPA promulgation of criteria or EPA approval of a state-promulgated criteria, that **new acute and chronic criteria for zinc** are in effect in Idaho by May 7, 2022 and are consistent with the discussion and analysis in the FWS Biological Opinion.[72] Initiation of consultation on new criteria was required by December 23, 2021. *EPA has failed to implement this RPA by the deadline and to date has not promulgated the RPA*.[73]

- Ensure, either through EPA promulgation of criteria or EPA approval of a state-promulgated criteria, that **new acute and chronic criteria for nickel** are in effect in Idaho by May 7, 2022 and are consistent with the discussion and analysis in the FWS Biological Opinion.[74] Initiation of consultation on new criteria was required by December 23, 2021. *EPA has failed to implement this RPA by the deadline and to date has not promulgated the RPA*.[75]

- FWS provided the same RPAs as NMFS for the **low-end hardness floor, arsenic, and mercury,** all of which can be found in the above discussion.[76]

Further, in acknowledgment that the final RPAs require Idaho and/or EPA to undergo rulemaking, the Services included interim RPAs to be implemented as a part of the CWA Section

[68] FWS Biological Opinion at 277. In the absence of specific data, the Service's best estimate of adequately safe cyanide concentrations for acute and chronic exposures, respectively, is 13 and 2.5 µg/L.
[69] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.a.
[70] FWS Biological Opinion at 278.
[71] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.a.
[72] FWS Biological Opinion at 282.
[73] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.a.
[74] FWS Biological Opinion at 283.
[75] *See* IDAPA 58.01.02, "Water Quality Standards" sec. 210.03.a.
[76] FWS Biological Opinion at 269. *See id*. at 285 (interim RPA for the low-end hardness floor), 272 (interim RPA for arsenic), 274 (interim RPA for *copper)*, 279 (interim RPA for mercury), and 280 (interim RPA for selenium).

402 NPDES permit process.[77] The intended purpose of the interim RPAs is to temporarily protect listed species until the rulemaking process is complete.[78] Each of the criteria for which the Services reached jeopardy determinations—with the exception of the hardness floor—was given final and interim RPAs. [79] In addition to each of these specific interim RPAs, FWS noted that EPA "consults with the Service over each new or reissued NPDES permit in Idaho to ensure that it will not cause jeopardy to the species or adverse modification to critical [] habitat." [80] It is unclear, however, the degree to which this consultation has been occurring.

The interim RPAs, along with EPA's authority to consult with the Services on NPDES permits, were only meant to "minimize any adverse effects during the implementation period *while new criteria [were] developed and adopted*."[81] The Services did not determine, in either Biological Opinion, that permanent use of the interim RPAs would be protective of listed species, even if EPA were to diligently apply them. Furthermore, in 2018, EPA authorized Idaho to administer the NPDES program in the state, making Idaho, not EPA, the agency responsible for writing and issuing NPDES permits.[82] As a NMFS employee noted in a 2018 email exchange, this shift in permitting authority raises questions regarding whether Idaho knows to, and is in fact, implementing the RPAs.[83] The exchange also noted concerns about the Service's lack of authority

[77] FWS Biological Opinion at 286.
[78] FWS Biological Opinion at 270.
[79] NMFS Biological Opinion at 282 (arsenic); FWS Biological Opinion at 271–72 (arsenic); FWS Biological Opinion at 279 (mercury); NMFS Biological Opinion at 284 (mercury); FWS Biological Opinion at 275–77 (cyanide); NMFS Biological Opinion at 284 (cyanide); FWS Biological Opinion at 278 (lead); FWS Biological Opinion at 281 (zinc); FWS Biological Opinion at 283 (nickel).
[80] *Id*. at 270.
[81] *Id*. (emphasis added).
[82] *See* Idaho NPDES Program Authorization, https://www.epa.gov/npdes-permits/idaho-npdes-program-authorization.
[83] E-mail from Johnna Sandow, Fish Biologist, NOAA Fisheries West Coast Region to Patricia Shaw-Allen, Ecotoxicologist, NOAA Fisheries (Oct. 10, 2018, 9:32 AM) (on file with author) ("I'm not sure Idaho

over the state.[84] In fact, there is none. But despite the change in NPDES permitting authority, EPA still bears the responsibility to carry out the RPAs. The transition underscores the importance of timely EPA action to do so.

Notably, FWS stated in its Biological Opinion that if the final RPAs are not completed by their effective dates, "all interim measures identified in the individual RPA shall be adopted as final for purposes of establishing aquatic life criteria in association with Idaho's water quality standards."[85] The meaning of this statement is unclear—i.e. whether the incorporation of the interim measures by rule would become the final RPA or whether their interim status continues until such date as the final RPAs are implemented. Regardless, EPA has failed and continues to fail to meet its obligation to implement the final RPAs, there is no assurance that the interim RPAs are being used by IDEQ in issuing NPDES permits, and the agencies did not find the interim RPAs adequate, in and of themselves, to avoid jeopardy and adverse modification of critical habitat.

The below tables set forth the final and interim RPAs included in FWS's and NMFS's Biological Opinions, the deadlines by which those RPAs were to be implemented, and the status of that implementation.

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understands that they need to implement those RPAs as they prepare permits for facilities discharging to waters with anadromous species/critical habitat.").

[84] E-mail from Patricia Shaw-Allen, Ecotoxicologist, NOAA Fisheries to Johnna Sandow, Fish Biologist, NOAA Fisheries West Coast Region (Oct. 10, 2018, at 6:39 AM) (on file with author).

[85] FWS Biological Opinion at 285.

Table 1: Outstanding NMFS Reasonable and Prudent Alternatives				
Element	**Risks to listed species at Idaho Criteria**	**RPA**	**Implementation Deadline[86]**	**Status as of 03/31/2022[87]**
Hardness Floor (General aspect)	- increase in mortality - decreases in growth and survival of juvenile salmonids NMFS Biological Opinion at 117.	Remove the low hardness floor on the hardness dependent metals criteria equations and instead calculate using actual site conditions. No interim measure. NMFS Biological Opinion at 281.	**5/7/2017**	Incomplete[88]
Arsenic	**Effects on listed snails:** - reduced food resources **Effects on listed salmonids** - reduced prey availability - reduced growth in juveniles - liver and organ damage - behavioral modifications - reduced reproductive success NMFS Biological Opinion at 124-25.	Adopt a new chronic criterion incorporating dietary exposure. Interim: Ensure that the 10 µg/L human health recreational use standard is applied. NMFS Biological Opinion at 282.	**5/7/2021**	Final RPA Incomplete[89] Interim Measure Unknown
Mercury	**Effects on salmonids:** - endocrine disruption - brain damage - behavioral abnormalities - reproductive impairment - reduced feeding efficiency and competitive ability NMFS Biological Opinion at 149-50.	Adopt a new chronic criterion for mercury. Interim: Use the 2001 EPA/2005 Idaho human health fish tissue criterion of 0.3 mg/kg wet weight. For water bodies for which fish tissue data are not available the water body will be presumed to meet the fish tissue criterion of 0.3 mg/kg wet weight if the geometric mean of measured concentrations of total mercury in water is less than 2 ng/L. If not, fish tissue data shall be collected. NMFS Biological Opinion at 284.	**5/7/2021**	Under Stipulated Order[90] **Draft by March 30, 2024**

*Additional Interim Measure: EPA to consult with NMFS over each new or reissued NPDES permit to ensure it will not cause jeopardy or adverse modification.[91]

[86] If EPA needed to initiate consultation about the new criteria, it was to have done so 135 days before the implementation deadline for each criterion. All deadlines retrieved from FWS Biological Opinion at 285.

[87] Idaho DEQ, *Water Quality Standards*, §210, https://adminrules.idaho.gov/rules/current/58/580102.pdf.

[88] DEQ believes that implementation of the interim measures identified in the Biological Opinion, along with implementation of other aspects of Idaho water quality standards, are sufficient to protect species listed under the ESA. Idaho DEQ, *2020 Triennial Review of Idaho Water Quality Standards*, 10. https://www2.deq.idaho.gov/admin/LEIA/api/document/download/15165.

[89] DEQ will prioritize adoption of a new arsenic standard when updates to EPA's 304(a) guidance are finalized. *Id*.

[90] *See* description of Stipulated Order, *supra* n.58.

[91] NMFS Biological Opinion at 286.

Table 2: Outstanding FWS Service Reasonable and Prudent Alternatives[92]				
Element	**Risks to listed species at Idaho Criteria**	**RPA**	**Implementation Deadline[64]**	**Status as of 03/31/2022[93]**
Cyanide	- Mortality - reduced growth - reduced swimming performance - reduced egg production - similar effects on prey. FWS Biological Opinion at 166-70.	Adopt new acute and chronic criteria using a temperature/toxicity correlation equation.[94] Interim: a zone of passage limited to no more than 25% of the volume of a stream must be maintained around any mixing zone for discharges that include cyanide. FWS Biological Opinion at 275-77.	**5/7/2021**	Incomplete
Lead	**For pulmonate Banbury Springs lanx snail:** - Reduced growth - Reduced egg production FWS Biological Opinion at 176.	Adopt a new chronic criterion. Interim: discharges must meet the chronic lead criterion at the end of pipe; no mixing zone is allowed. FWS Biological Opinion at 278.	**5/7/2023**	Incomplete
Nickel	**For listed snail species:** - Mortality - negative effects to reproduction, numbers, and distribution FWS Biological Opinion at 218-19.	Adopt new acute and chronic criteria. Interim: the mixing zone for discharges of nickel into snail habitat must be limited to no more than 25% of flow. No mixing zone for discharges into lanx habitat. FWS Biological Opinion at 283.	**5/7/2022**	Incomplete
Zinc	**For the bull trout:** - impaired ability of habitat to provide for normal reproduction, growth, and survival. **For the Kootenai River white sturgeon:** - reduced growth and survival - impeded reproduction and maintenance or increase of the wild population. - impaired ability of critical habitat to provide for normal behavior, reproduction, and survival. FWS Biological Opinion at 204, 207-08.	Adopt new acute and chronic criteria. Interim: maintain a zone of passage sufficient to allow unimpeded passage of adults and juveniles. Zone must be limited to less than or equal to 25% of the volume of the stream. FWS Biological Opinion at 281-82.	**5/7/2022**	Incomplete

[92] FWS incorporated the NMFS RPAs into its Biological Opinion. The duplicative RPAs are not included in the above table.
[93] Idaho DEQ, *Water Quality Standards*, §210. https://adminrules.idaho.gov/rules/current/58/580102.pdf
[94] In the absence of specific data, the Service's best estimate of adequately safe cyanide concentrations for acute and chronic exposures, respectively, is 13 and 2.5 µg/L. FWS Biological Opinion at 277.

IV. ESA-listed Species are Likely to be Harmed Absent Implementation of the RPAs.

As stated above, RPAs are rare and only occur when the Services determine that an agency action jeopardizes ESA-listed species or risks destruction or adverse modification of its critical habitat. If EPA continues to fail to implement the RPAs, it is subjecting listed species to the very conditions that caused the Services to make jeopardy decisions in the first place. EPA's failure to implement the RPAs can, therefore, result in significant harm to listed species. The below sections address the harm posed to Idaho's listed species due to EPA's failure to implement the RPAs.

A. Low Hardness Floor

Water hardness is defined by the amount of dissolved minerals, primarily calcium and magnesium, in water.[95] Some metals criteria proposed by IDEQ are hardness-dependent, "meaning that rather than establishing a criterion as a concentration value, the criteria are defined as a mathematical equation using the hardness of the water as the independent variable."[96] The criteria that vary based on site-specific hardness are the following: cadmium, copper, chromium III, lead, nickel, silver, and zinc.[97] Instead of using site-specific water hardness for determining criteria, IDEQ proposed a "low-hardness floor" of 25 mg/L, meaning that the lowest number used in calculating criteria would be 25 mg/L, even if the actual hardness were much lower. The use of a hardness floor of 25 mg/l in calculating acceptable levels of metals allows an increased exposure of listed fish to levels that result in adverse effects, ranging from a direct increase in mortality to decreases in growth and survival of NMFS-protected juvenile Snake River spring/summer Chinook salmon, Snake River fall Chinook salmon, Snake River Sockeye salmon and Snake River

[95] U.S. Geological Survey, Water Science School, Science, Hardness of Water, https://www.usgs.gov/special-topics/water-science-school/science/hardness-water#overview
[96] NMFS Biological Opinion at 102.
[97] *Id*.

Basin steelhead,[98] and FWS-protected species bull trout, Kootenai River white sturgeon, and Banbury Springs lanx.[99]

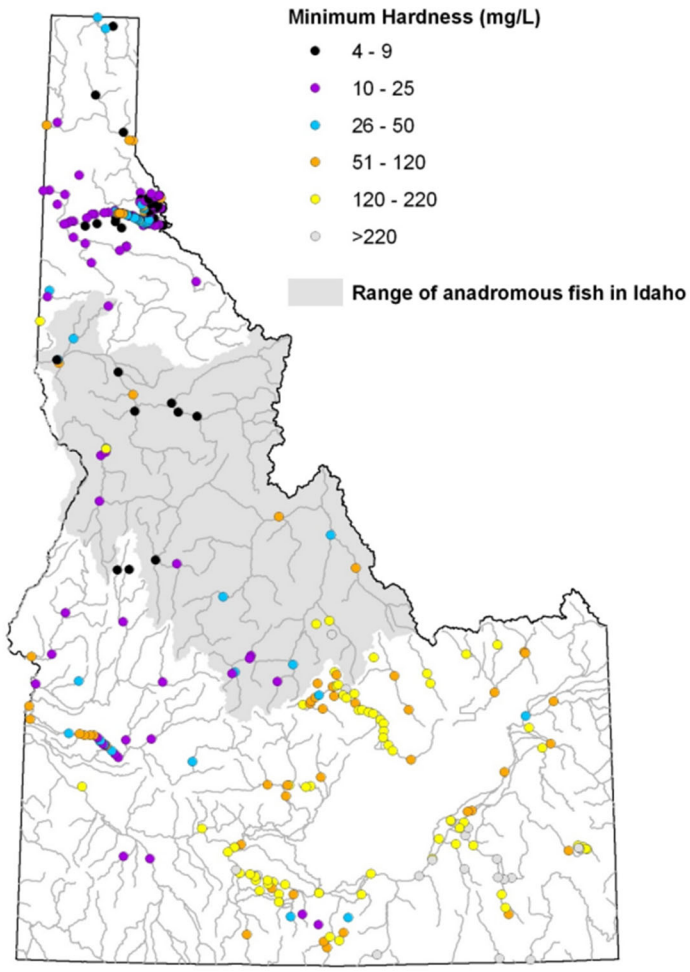
Fish maintain their internal mineral balance through osmoregulation, and at lower hardness levels (aka soft water), the energy required to maintain that balance can be high.[100] Devoting energy to this task can lead to reduced growth, reduced swimming ability, and reduced ability to recover from severe exercise when compared to fish in hard water.[101] Furthermore, as hardness decreases and it becomes more energy intensive for fish to maintain homeostasis, fish may simultaneously become more sensitive to metals that inhibit ionoregulation.[102] Fish such as salmonids, which migrate throughout their lifetimes, may be even more susceptible to changes in water hardness and metal toxicity. For example, a 2010 study of rainbow trout demonstrated that fish acclimated to or incubated in soft water may continue to experience increased sensitivity to metal toxicity even after the fish move into higher hardness water.[103] According to the NMFS Biological Opinion:

> This has implications for salmonid life histories and habitats. Water hardness tends to be lowest near the headwaters of streams and increase downstream, and some salmonids tend to ascend streams to spawn in the upper reaches of watersheds and after emerging, their fry move downstream into higher hardness waters.[104]

Within the range of listed salmon or steelhead, water hardness tends to decrease from south to north and can be highly variable with values as low as 4 mg/L having been measured in soft water

[98] *Id*. at 117.
[99] FWS Biological Opinion at 275 (copper and hardness floor), 278 (lead and hardness floor), 283 (nickel and hardness floor).
[100] *Id*. at 105.
[101] *Id*.
[102] *Id*. at 106.
[103] *Id*.; *see also*, Mebane, C.A., D.P. Hennessy, and F.S. Dillon. 2010. Incubating rainbow trout in soft water increased their later sensitivity to cadmium and zinc. *Water, Air, and Soil Pollution*. 205(1-4): 245–250.
[104] NMFS Biological Opinion at 106–107.

areas.[105] "[H]owever, the true minimum hardnesses [sic] in streams in granitic watersheds are probably close to that of snowmelt, which is in the range of 0.5 to 1 mg/L total hardness."[106] The figure below shows minimum hardness values measured at 323 sites in Idaho between 1979 and 2004.[107]



There are, as the figure indicates, many waters in Idaho with hardness levels below IDEQ's proposed hardness floor. Calculating criteria for metals that are hardness dependent using the

[105] *Id*. at 112.
[106] *Id*.
[107] *Id*. at 114.

proposed hardness floor of 25 mg/L instead of the true water hardness can therefore result in hardness dependent metals at levels in listed species' habitats that are not protective.

B. Zinc

Zinc criteria are hardness dependent, as described above.[108] If the criteria were calculated with the proposed hardness floor of 25 mg/L the acute and chronic criteria would be 35 and 32 µg/L respectively.[109] A 2012 study on rainbow trout found that at 36 µg/L zinc in water with a hardness value of 7 mg/L, 80 percent of the trout were killed.[110] Furthermore, NMFS determined that:

> Increased levels of zinc over natural body concentrations can result in mortality, growth retardation, histopathological alterations, respiratory and cardiac changes, and inhibition of spawning and many other elements critical to fish survival. Exposure to high zinc concentrations can result in damage to the gills, liver, kidney and skeletal muscle and cause a physiological shift to occur, making gas exchange more difficult."[111]

Salmonids appear to have varying sensitivity zinc at different life stages with the greatest effects occurring during the first two months after hatching.[112] The majority of the information shows that in waters with a hardness less than about 25 mg/L, the proposed criteria would not be sufficiently protective of listed Snake River salmon and steelhead if they were exposed at their most sensitive life stages.[113]

The proposed zinc criteria are also likely to cause mortality of juvenile bull trout and reduce bull trout prey abundance.[114] One study of the proposed acute criterion for zinc found substantial

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[108] NMFS Biological Opinion at 187, 193.
[109] The acute and chronic criteria are nearly the same presumably because zinc is a "fast-acting toxicant that is no more toxic in long-term exposures than in short-term exposures." FWS Biological Opinion at 197.
[110] NMFS Biological Opinion at 108.
[111] *Id.*
[112] *Id*. at 193; *see also* FWS Biological Opinion at 202.
[113] *Id*. at 193.
[114] FWS Biological Opinion at 266.

mortality rates to larger juvenile fish at low water hardness values with a pH of 7.5.[115] In Idaho, waters occupied by bull trout with similar hardness and pH to those from the study are common.[116] Resident (non-anadromous) and juvenile migratory bull trout prey on terrestrial and aquatic insects, macro-zooplankton, and small fish, while adult bull trout are piscivores.[117] Some information suggests that elevated zinc concentrations could cause measurable losses of bull trout prey insect species.[118] One study found that zinc concentrations just slightly above the proposed criteria level decimated the mottled sculpin, a forage fish prey species.[119] IDEQ's proposed zinc criteria are also likely to cause mortality of Kootenai River white sturgeon and cause sub-lethal effects to normal sturgeon behavior, resulting in decreased reproduction and survival and causing reductions in their prey species.[120] As with bull trout and salmonids, white sturgeon show greater sensitivity to zinc at earlier life stages.[121] A test on Columbia River white sturgeon showed that "the apparent threshold for adverse effects of zinc to white sturgeon was the [Idaho acute] criterion concentration [that] indicates the potential for adverse effects from short-term exposures of zinc to a sensitive life stage of white sturgeon."[122] The proposed zinc criteria are also likely to adversely affect freshwater mussels, a major food item for white sturgeon, as well as algae and diatoms, which in turn would cause a loss of herbivore species that are also prey for the sturgeon.[123] "Reduced prey availability would mean reduced sturgeon body weight, increased energy expenditure to procure prey, decreased energy available for reproduction, and generally reduced

[115] *Id*. at 203.
[116] *Id*.
[117] *Id*. at 203–04.
[118] *Id*. at 204.
[119] *Id*.
[120] *Id*. at 267.
[121] *Id*. at 206.
[122] *Id*.
[123] *Id*.

survival."[124]

C. Nickel

Nickel criteria are also hardness dependent.[125] At water hardness values of 10, 25, 50, 100, and 250 mg/L, the acute nickel criterion value is 67, 145, 260, 468, and 1017 µg/L, respectively, while the chronic criterion values are 7, 16, 29, 52, and 113 µg/L, respectively.[126] As noted above, the proposed action uses a hardness floor when calculating metal criteria that "presumes that at a water hardness of 10 mg/L, nickel is no more toxic than at a water hardness value of 25 mg/L."[127] FWS did not find any evidence supporting this presumption, and in fact found evidence to the contrary for nickel.[128] One 2007 study using water fleas found that sensitivity to nickel decreased as water hardness increased.[129]

Exposure to nickel at the proposed acute and chronic criterion likely jeopardizes the Banbury Springs lanx.[130] FWS found that the proposed acute criterion is likely to cause "severely retarded growth" to the species while the proposed chronic criterion is likely to cause mortality and population reductions.[131] A 2013 study identified the 96-hour lethal concentration to 50 percent of snails exposed to nickel at 445 µ/L at a water hardness of 85 mg/L, similar to the proposed acute criterion of 408 µ/L.[132] A 2010 laboratory study on snails from the same family as the Banbury Springs lanx found that exposure to nickel at 1.6 µg/L for 21 days in waters with a

[124] *Id*.
[125] NMFS Biological Opinion at 163.
[126] FWS Biological Opinion at 214.
[127] *Id*.
[128] *Id*.
[129] *Id*. at 215.
[130] *Id*. at 219, 258.
[131] *Id*.
[132] *Id*. at 215.

hardness value of 212 mg/L led to adverse effects for 20 percent of the population.[133] At the same water hardness, the proposed Idaho nickel chronic criterion would be 98 µg/L, "which indicates that the proposed chronic water quality criterion for nickel would be severely underprotective of [Banbury Spring lanx]."[134] A second laboratory study in 2014 revealed similar adverse effects at levels well below the proposed chronic criterion.[135] Finally, a 2011 study testing the "effects of long-term nickel exposures to complex pond-like communities" found a slight decline in snail abundance at 24 µg/L and, significantly, that snail species were completely extirpated at exposures of 48 and 96 µg/L.[136] "The 48 µg/L treatment with extirpated snails was almost the same nickel concentration as the IDEQ proposed chronic aquatic life criterion of 52 µg/L (tests waters had mean hardness of 100 mg/L, dissolved organic carbon of 3.8 mg/L, and pH of 8.6.)."[137] On this basis, FWS concluded that the proposed acute and chronic criteria for nickel are likely to jeopardize the Banbury Springs lanx throughout its range.

D. Lead

The proposed chronic criterion for lead is hardness dependent.[138] At water hardness values of 10, 25, 50, 100, and 250 mg/L, the chronic criterion values for lead are 0.2, 0.5, 1.2, 2.5, and 6.7 µg/L, respectively.[139] As discussed above, the proposed IDEQ criteria provide for a hardness floor of 25 mg/L that "presumes that at a hardness value of 10 mg/L, lead is no more toxic than at a hardness of 25 mg/L."[140] However, FWS did not find any scientific evidence to support this

[133] *Id*. at 216.
[134] *Id*.
[135] *Id*. at 217.
[136] *Id*. at 218.
[137] *Id*.
[138] FWS Biological Opinion at 171.
[139] *Id*.
[140] *Id.*

assumption and did not rely on it for its analysis.[141]

The proposed chronic criterion for lead is likely to jeopardize the Banbury Springs lanx by adversely affecting growth and egg production.[142] The Banbury Springs lanx is a pulmonate snail considered to be in the family Lymnaeidae.[143] "Pulmonate snails in the family Lymnaeidae have been shown to be hypersensitive to chronic lead toxicity."[144] The reasons for this hypersensitivity appear to be related to the high demand for calcium by juvenile pulmonate snails, relative to their body size and the role of lead in mimicking and disrupting calcium uptake." [145] One study demonstrated a 20 percent reduction in growth of juvenile snails when exposed to a dissolved lead concentration of about 3 µg/L in water with a hardness of about 102 mg/L.[146] A second study that tracked egg production as a measure of reproductive output found that snails exposed to 1 µg/L lead in water with a hardness value of 87 mg/L experienced reduced egg production.[147] The proposed chronic criterion at that hardness value is 2.2 µg/L.[148] That same study estimated that a no-effect concentration of lead would be 0.4 µg/L, less than one fifth the amount allowed by the proposed criteria.[149] Because of the hypersensitivity of pulmonate snails in the family Lymnaeidae, the proposed chronic lead criterion is likely to adversely affect the Banbury Spring lanx.

E. Arsenic

For arsenic, the primary concern for ESA-listed species is from bioaccumulation through

[141] *Id*.
[142] *Id.* at 176, 258.
[143] *Id*. at 172.
[144] *Id*.
[145] *Id.*
[146] *Id*.
[147] *Id.* at 174.
[148] *Id*.
[149] *Id.*

the food chain.[150] For listed snail species, the proposed arsenic levels are likely to significantly impact algal communities, thereby reducing the availability of a significant food resource throughout the snails' habitat ranges.[151] Studies have shown that exposure to arsenic at levels as low as 22 µg/L can impair photosynthesis in algal communities by 50 percent.[152] For fish, studies conducted using rainbow and bull trout have demonstrated that arsenic ingestion at levels below the proposed water quality criterion are associated with liver and other organ damage, reduced growth in salmonid juveniles, and adverse physiological effects.[153] Specifically, arsenic at proposed criterion levels is likely to cause reduced growth and survival, organ damage, and behavioral modifications to bull trout species.[154] For the Kootenai River white sturgeon, exposure to arsenic is likely to cause altered feeding behavior, and reduced body weight, prey availability, reproductive success, and survival.[155] NMFS's review of waterborne arsenic concentrations in Idaho and Montana waters suggested that, through bioaccumulation, concentrations harmful to salmonids may even occur in streams with dissolved arsenic concentrations on the order of 10 µg/L or less.[156] The chronic criterion proposed by IDEQ was 150 µg/L. Furthermore, Idaho's proposed criterion is based on dissolved arsenic, which evidence suggests is less of a concern than

[150] The majority of studies and literature suggest that waterborne exposure to arsenic at concentrations near the proposed standard does not affect salmonids (NMFS Biological Opinion at 118), although one study suggested that arsenic concentrations of 42 to 134 µg/L were estimated to be associated with the onset of embryo mortality (lethal concentrations killing 1% to 10% of tested fish). FWS Biological Opinion at 143. However due to reporting issues, that study could not be critically reviewed.
[151] FWS Biological Opinion at 140, 260.
[152] *Id*. at 142.
[153] NMFS Biological Opinion at 119–20; FWS Biological Opinion at 143–44.
[154] FWS Biological Opinion at 260.
[155] *Id*. at 261.
[156] NMFS Biological Opinion at 120; FWS Biological Opinion at 144.

particulate arsenic, which is more important as a source to aquatic food webs.[157] In other words, the proposed arsenic criterion may not protect against levels of particulate arsenic, including protection of sediment quality that is key to protecting species from arsenic.[158] Both NMFS and FWS agree "that the dissolved arsenic criterion may be less relevant than a sediment, dietary, or tissue residue based criterion."[159]

The arsenic RPAs assumed that most of Idaho waters are subject to human health criteria of 10 µg/L.[160] However, EPA disapproved Idaho's 10 µg/L criteria for protection of human health in 2016 following a lawsuit by NWEA.[161] As part of that same disapproval action, EPA also disapproved Idaho's 1999 adoption of 50 µg/L criteria for human health.[162] Neither Idaho nor EPA has yet promulgated a new arsenic criterion, and in the interim EPA has recommended that Idaho use its narrative criteria to apply EPA's far more stringent 304(a) criteria.[163] Idaho has not taken this position, and instead considers the disapproved 10 µg/L to continue to be the effective criteria.[164] While the water quality standards are ambiguous as to whether these criteria are based on dissolved or total recoverable arsenic, the Services presumed the latter to be the case as

[157] NMFS Biological Opinion at 120–21; FWS Biological Opinion at 146. "Dissolved" metals are those that remain after a water sample is passed through a 0.45 µm filter, are truly in solution, and will not settle from gravity. Particulate metals are larger and are subject to settling due to gravity. NMFS Biological Opinion at 85–86.
[158] FWS Biological Opinion at 148.
[159] *Id*.
[160] *Id*. at 151.
[161] *See* Letter from Daniel Opalski, Director Office of Water and Watersheds, EPA Region 10 to Barry Burnell, Water Quality Program Administrator, IDEQ, Re: EPA Disapproval of Idaho's Arsenic Human Health Water Quality Criteria (Sept. 15, 2016).
[162] *Id.*
[163] *Id*. at 5.
[164] IDEQ, EPA Actions on Proposed Standards, https://www.deq.idaho.gov/water-quality/surface-water/epa-actions-on-proposed-standards/ ("10 µg/L continues to be the CWA effective arsenic criterion for both exposure through fish consumption only and exposure through drinking water+fish consumption").

"[n]either swimmers nor fish can be expected to filter their water prior to ingestion."[165] FWS then evaluated whether this assumed criterion would protect listed species, concluding that, "[w]hile it is much lower than the proposed chronic criterion, in some field settings, adverse effects to fish, or at least elevated arsenic in prey organisms, were reported from locations where the 10 µg/L criterion was only slightly exceeded."[166] However, FWS ultimately concluded that 10 µg/L would likely be protective because harmful concentrations of inorganic arsenic are not usually associated with ambient levels below 10 µg/L.[167] Regardless, EPA must implement the RPA for arsenic if for no other reason than the fact that Idaho has no EPA-approved human health criteria on which the Services relied and EPA has repeatedly sought extensions to provisions of its Consent Decree that require EPA or IDEQ action to adopt new human health criteria.[168]

To summarize, if only direct water exposures were considered, arsenic would be of minimal concern to listed salmonids. However, through bioaccumulation, arsenic concentrations below the chronic criterion of 150 µg/L and even below the purported human health-based criterion of 10 µg/L have been observed to cause harm to salmonids. As such, adverse effects are likely to occur at the chronic criterion via food web transfer.[169]

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[165] *Id*; FWS Biological Opinion at 138.
[166] FWS Biological Opinion at 151.
[167] FWS Biological Opinion at 156.
[168] Consent Decree *Northwest Environmental Advocates v. EPA*, Case 3:15-cv-01151-HZ (June 7, 2016), ¶ 6 ("If EPA signs proposed new arsenic criteria for Idaho by November 15, 2018, and Idaho does not adopt replacement criteria that EPA approves by July 15, 2019, EPA will sign a notice of final rulemaking action on EPA's proposed arsenic criteria for Idaho by July 15, 2019."); U.S. District Court District of Oregon (Portland (3)) CIVIL DOCKET FOR CASE #: 3:15−cv−01151−HZ ("ORDER: Granting Motion 22. The Consent Decree shall be modified by extending the Paragraph 5 deadlines to November 15, 2022, and the Paragraph 6 deadlines to November 15, 2023. Ordered by Judge Marco A. Hernandez. (jp) (Entered: 06/15/2018)"); ("ORDER: Granting Defendant's Unopposed Second Motion to Modify Consent Decree 24. The Consent Decree shall be modified by extending Paragraph 5 deadlines to November 15, 2023 and the Paragraph 6 deadlines to November 15, 2024. Ordered by Judge Marco A. Hernandez. (jp) (Entered: 06/21/2022)").
[169] NMFS Biological Opinion at 124–25.

F. Cyanide

The proposed acute and chronic criteria for cyanide are 22 µ/L and 5.2 µ/L, respectively.[170] The toxicity of cyanide is strongly influenced by water temperature with increased toxicity occurring at lower temperatures.[171] Despite that low temperature waters "c[an] hardly be considered 'unusual'" in Idaho, the proposed criteria make no adjustment to account for this increased toxicity.[172] In fact, Idaho water temperatures are, on average, below 6° C throughout the winter months.[173] NMFS considers an acute criterion protective when exposure to a species at the final acute value (the criterion multiplied by two) causes less than 50 percent of a population to die.[174] One study on rainbow trout by Kovacs and Leduc demonstrated that a lethal concentration of 50 percent occurred after four days of exposure to cyanide at 27 µg/L and 40 µg/L in 6° C and 12° C waters, respectively.[175] The acute cyanide criterion, therefore, is not protective of listed salmonids. One study of the chronic cyanide toxicity effects on juvenile rainbow trout observed reduced growth to exposures of 5µg/L of cyanide at 6° C.[176] The same researchers also observed reduced swimming ability in rainbow trout after a 20-day exposure to 5µg/L of cyanide.[177] These studies demonstrate the inadequacy of the 5.2 µg/L chronic cyanide criterion.

The proposed acute and chronic criteria for cyanide are likely to jeopardize bull trout and adversely modify its critical habitat.[178] Relying on the same studies as NMFS, FWS found that

[170] NMFS Biological Opinion at 139.
[171] *Id*.
[172] FWS Biological Opinion at 166.
[173] NMFS Biological Opinion, at 141, Fig. 2.4.5.1.
[174] NMFS Biological Opinion at 142.
[175] *Id.*
[176] *Id.* at 143.
[177] *Id.*
[178] FWS Biological Opinion at 168–70, 258.

substantial mortality of exposed bull trout likely to occur at the proposed acute criterion.[179] Regarding the proposed chronic criterion of 5.2 µg/L, FWS cited a study showing long-term exposure at a cyanide concentration of 5.6 µg/L caused an 18 percent reduction in egg production for brook trout, a species closely related to bull trout. In cold temperatures, reduced growth and swimming performance in rainbow trout were observed at concentrations less than 4.8 µg/L.[180] In addition to causing these adverse effects, FWS determined "[t]he proposed acute and chronic criterion are likely to create lethal or sublethal chemical barriers that impair or preclude bull trout migration [] and movement between various types of habitats."[181] Migration is essential to the species' survival. Additionally, exposure to cyanide levels at the proposed criteria cause adverse effects to bull trout prey species.[182] A decline in prey affects the bull trout's ability to maintain robust populations, and is therefore likely to adversely affect the species.[183]

FWS determined likely jeopardy and adverse modification of critical habitat for the Kootenai River white sturgeon for the same reasons as for the bull trout as well as two others.[184] First, one study of the bluegill found that spawning was "completely inhibited at a concentration of 5.2 µg/L [hydrogen cyanide]," the same concentration as Idaho's proposed chronic criterion, "which clearly indicates that the criteria cannot be considered fully protective of critical life functions in all fish species."[185] Second, FWS determined that sediment-sorbed cyanide posed a risk to white sturgeon eggs and early life stage juveniles in sturgeon critical habitat: "Sediment

[179] *Id.* at 166.
[180] *Id.*
[181] *Id.* at 169.
[182] *Id.*
[183] *Id.*
[184] *Id*. at 170.
[185] *Id*. (internal quotations omitted).

quality is critically important to the health of white sturgeon critical habitat because all life stages of the sturgeon are extensively exposed to sediments, either through dermal contact (all life stages) or through incidental ingestion while feeding (juveniles and adults)."[186]

Thus, neither the proposed acute nor chronic cyanide criteria are protective for listed salmonids, bull trout, the Kootenai River white sturgeon, or the critical habitats of the bull trout and white sturgeon.

V. Relief Requested by This Petition and Conclusion

EPA has yet to implement all the final RPAs set forth in the 2014 NMFS and 2015 FWS Biological Opinions. In order to protect the ESA-listed species covered by the Biological Opinions from jeopardy and to prevent adverse modification or destruction of those species' critical habitats—and to avoid "take" of these species—EPA must implement these RPAs. Therefore, for the reasons detailed above, NWEA hereby petitions EPA to promulgate rules consistent with the outstanding final RPAs set forth in the May 7, 2014 NMFS Biological Opinion and the June 15, 2015 FWS Biological Opinion (see Tables 1 & 2, *supra* pp. 15–16), with the exception of mercury as EPA is under a stipulated order regarding criteria for that pollutant.[187] Through such rulemaking, EPA would adopt new or revised aquatic life criteria for the State of Idaho, as follows:

- Hardness floor: remove the low hardness floor on the hardness dependent metals criteria equations and instead calculate using actual site conditions
- Arsenic: adopt a new chronic criterion incorporating dietary exposure
- Cyanide: adopt new acute and chronic criteria using a temperature/toxicity correlation equation
- Lead: Adopt a new chronic criterion

[186] *Id.*
[187] *See supra* n.58.

- Nickel: Adopt new acute and chronic criteria
- Zinc: Adopt new acute and chronic criteria

Given that EPA has missed the existing deadlines for implementation of these RPAs, and the fact that—as determined by NMFS and FWS—implementation is necessary to avoid jeopardy to ESA-listed species, NWEA further requests that EPA make a final decision on this Petition within sixty (60) days of receipt. If EPA grants the Petition, NWEA requests that EPA propose the new or revised aquatic life criteria within one (1) year of the date of receipt of this Petition and that EPA thereafter promptly finalize the proposed criteria.

Respectfully submitted,

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Northwest Environmental Advocates
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Dated this day, the 1st of June, 2023.

Enclosed:

Attachment A: National Marine Fisheries Service, Final Endangered Species Act Section 7 Formal Consultation and Magnuson-Stevens Fishery Conservation and Management Act Essential Fish Habitat Consultation for Water Quality Toxics Standards for Idaho, NMFS No. 2000-1484 (May 7, 2014)

Attachment B: Fish and Wildlife Service, Biological Opinion for the Idaho Water Quality Standards for Numeric Water Quality Criteria for Toxic Pollutants, OlEIFW00-2014-F-0233 (June 15, 2015)