A TOXICS-FOCUSED BIOLOGICAL OBSERVING SYSTEM FOR PUGET SOUND

Developed by the Washington Department of Fish and Wildlife and NOAA Fisheries for the Puget Sound Partnership

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Executive Summary

As a result of human activities, our nation's estuaries are the recipients of a wide range of toxic chemical contaminants. Concern about the possible effects of these substances has led to the implementation of toxics monitoring programs in some major estuaries. Much of this monitoring is focused on water and sediments, with sediments viewed as repositories for many toxics entering aquatic ecosystems. However, there is increasing evidence that we are missing important ecological impacts by simply monitoring water and sediments, and neglecting biota. We now realize that toxics affect biota throughout the ecosystem, including humans. As a result, biologically-based monitoring, at multiple trophic levels, is increasingly recognized as an important component of efforts to protect estuaries from toxic chemicals (Karr 2006).

Why is Biological Monitoring Important in Puget Sound?

Biological monitoring identifies problems associated with toxics in the Puget Sound ecosystem.

In Washington State, freshwater, estuarine, and marine habitats are protected from the impacts of toxic contaminants largely through legislation that focuses on maintaining water and sediment quality. Measuring contaminant concentrations in regional sediments and waters to determine their compliance with these standards is important to reduce toxic inputs into Puget Sound. However, research findings increasingly indicate that measuring contaminant levels in the physical environment is not enough. For example, it is becoming increasingly clear that profiling sediment contamination alone may not provide a complete picture of the impacts of toxicants on the Puget Sound ecosystem. Recent surveys of sediment contamination in the Sound, conducted as part of the Puget Sound Assessment and Monitoring Program (PSAMP), suggest that only a relatively small proportion of Puget Sound sediments are contaminated, based on Washington State Management Standards, is relatively small (Long et al. 2005). This might be interpreted to mean that persistent, sediment-associated contaminants are not a major problem for aquatic life in most of Puget Sound. However, monitoring has uncovered unexpected impacts of toxics on Puget Sound biota that were not predicted from abiotic measures. These include:

- **Contamination of the pelagic food web.** This is indicated by findings such as high levels of persistent bioaccumulative toxics in Pacific herring, a pelagic fish species that has very little contact with sediments and benthic prey (West et al. 2008), in juvenile and adult Pacific salmon (Stein et al. 1995; Stehr et al. 2000; O'Neill and West 2009), and in mussels suspended in the water column (PSAT 2007a; Kimbrough et al. 2008).

- **Emerging chemicals of concern, often not detected in water or sediments, are present in Puget Sound biota.** A notable example are the flame retardants, polybrominated diphenyl ethers or PBDEs, that have been identified in marine mammals, marine fish, and salmon from Puget Sound (Krahn et al. 2007; O'Neill et al 2007; PSAT 2007a; Ylitalo et al 2007; Sloan et al. 2009). Concentrations are relatively high in some populations, in spite of the fact that these chemicals are typically found at relatively low concentrations in Puget Sound sediments (Dutch and Aasen 2007; Dutch and Weakland 2009). Additionally, juvenile salmon and benthic flatfish are showing evidence of exposure to endocrine-disrupting compounds (EDCs), including estrogenic substances that are only beginning to be measured in water and sediments in Puget Sound (King County 2007). The estrogen-dependent yolk-protein, vitellogenin, which is normally only produced in maturing female fish, has been detected in both male flatfish and juvenile salmon from several sites in Puget Sound (Johnson et al. 2008, Peck et al. 2009). In flatfish, abnormal vitellogenin production appears to be associated with altered reproductive timing (Johnson et al. 2008).

- **Storm water effects on Puget Sound biota.** Mixtures of contaminants in storm water are associated with a surprisingly high degree of pre-spawn mortality (PSM) among adult coho salmon in many lowland urban streams, with overall die-offs that generally range from ~ 20% to 90% of the fall coho runs in urban drainages (McCarthy et al. 2008, Scholz et al. submitted). By comparison, premature coho spawner die-offs in non-urban (e.g., forested) drainages are low. This phenomenon would not have been predicted from measurement of individual pollutants alone, as concentrations of individual toxics in urban runoff are generally lower than those that those that would be expected to be acutely lethal to adult salmon based on laboratory toxicity studies.

- **Adverse effects in biota at contaminant concentrations considered safe under sediment management standards**. Sediment management standards for Washington State, which were developed from effects of toxicants on small, benthic invertebrates, are not always protective of fish and wildlife. For example, at sediment total PAH concentrations well below the "no effects level" under Washington State's Sediment Quality guidelines, bottomfish commonly exhibit liver disease, reproductive impairment, and altered growth (Johnson et al. 2002). Moreover, spawned herring eggs at some Puget Sound sites can accumulate concentrations of PAHs well above threshold levels associated with egg and larval toxicity, in areas where sediment PAH levels are considered acceptable (PSAT 2007a, Incardona et al. 2009).

The problems described above would not have been identified unless scientists had been monitoring concentrations and effects of toxic contaminants in Puget Sound biota in addition to monitoring abiotic matrices such as water and sediments. Clearly, biological monitoring is needed if we hope to understand and remedy the problems created by toxic compounds in the Puget Sound Ecosystem. "The health of Puget Sound" is an inherently biological concept, and the most directly relevant way to monitor the health of the Sound is to pay attention to the biology of the system.

Biological monitoring can inform our toxics reduction efforts in several important ways.

As mentioned above, the biota are our best sentinels for the impacts of toxic contaminants in the Sound, and they are also our best indicators of whether or not our management actions (e.g., loadings reductions, land use changes) are successful. When chemical concentrations and toxic injury decline in response to our cleanup activities, we have strong evidence that they have been effective (Myers et al. 2008). Biota can also provide information about the location, geographic extent, and severity of the problem. By paying close attention to aquatic species, we can tell whether a problem is associated with urban hot spots, or with broad and diffuse contamination that will be more difficult to control. This information can then be used to prioritize our toxics reduction efforts and select the most appropriate locations for monitoring studies or management actions. For example, biological effects such as liver lesions in flatfish and pre-spawn mortality in coho salmon show strong associations with urban land use and industrial activities (Myers et al. 2003; McCarthy et al. 2008; Scholz et al. submitted), making it possible to target specific watersheds for more detailed evaluations of loadings, toxics reduction activities, and monitoring. On the other hand, mercury concentrations in Puget Sound fish are similar throughout the Sound, with age being the most important factor in determining body burdens (West et al. 2001, PSAT 2007a). This suggests that mercury contamination is more diffuse, so monitoring and modeling loadings of mercury in specific watersheds to identify toxics reduction activities might not be such an effective strategy. The biota can also tell us when and how often to monitor, allowing us to target time points when species of concern will be present.

Biological monitoring is key to ecosystem management.

Monitoring biota allows us to evaluate the impacts of toxics – and the effectiveness of our efforts to reduce them - in an ecosystem context. Collecting toxicant exposure data in combination with other biological data on a broad range of species can give us a better sense of how contaminants may affect the resilience of the system. We can understand more about the potential for recovery of specific species and important ecosystem functions; we can better evaluate the impacts of toxics coupled with other stressors, and their potential indirect effects through the food web.

What types of biological monitoring are currently being done in Puget Sound?

As part of the PSAMP, concentrations of contaminants and their effects are being monitored in selected species. Ongoing efforts include measurement of contaminants in a few species of fish, marine mammals, and marine birds; liver disease in English sole; and benthic community structure and sediment toxicity to invertebrates in bioassays. While this is valuable work, it does not constitute a comprehensive, ecosystem-wide program. The species involved are fairly limited, important trophic relationships and food-web linkages are not included, only a few biological effects are measured, and efforts carried out on different species by different agencies are not always well integrated.

A mandate for improved toxics monitoring and reduction

The Washington State statute that established the Puget Sound Partnership (PSP) declares that a significant reduction of toxics that enter the Puget Sound’s fresh and marine waters is necessary to achieve the recovery of Puget Sound (Engrossed Substitute Senate Bill 5372; PSP 2007). A part of this agenda is “to develop plans for improved surveys of toxic contaminants and their effects, including effects on human health and the biological organisms of the Puget Sound ecosystem". The PSP further recommends that "scientists from government and elsewhere should collaborate in an integrated, comprehensive program of scientific investigation to characterize conditions and stressors, [and] test hypotheses relevant to toxics harm and control". To assist in carrying out these objectives, we recommend that biologically-based ecosystem-wide monitoring for toxics should be implemented in Puget Sound, through a program that we call a toxics-focused biological observing system (TBiOS).

What is TBiOS?

The TBiOS is a biologically based framework for toxics monitoring and research that assesses exposure and effects of chemical contaminants in biota, integrated across ecologically relevant habitats and food webs. It includes three major components:

1. Region-wide monitoring to document large-scale geographical and long-term temporal trends in contaminants in the Sound;

2. Localized effectiveness monitoring in areas where toxics reduction efforts have occurred. One example of such a study is the monitoring conducted by NOAA, EPA, and the Army Corps of Engineers in Eagle Harbor on Bainbridge Island following the closure of the Wyckoff creosote plant at that site, and subsequent cleanup and capping of PAH-contaminated sediments (Myers et al 2008). Prior to capping, studies had shown high PAH exposure and high prevalences of PAH-associated liver lesions in resident English sole. After source control and capping, exposure levels and liver lesion prevalences in Eagle Harbor English sole gradually declined, reaching levels comparable to those found at reference sites in approximately five years.

3. Diagnostic research studies assessing exposure and effects of chemical contaminants in biota. A recent example is a joint WDFW/NOAA study on the effects of PAHs on herring embryos (Incardona et al. 2009). This study showed that Pacific herring embryos exposed to PAHs in the laboratory developed heart abnormalities such as cardiac arrhythmia at concentrations within the range of those observed in herring embryos collected from sites in Central Puget Sound.

The TBiOS will maintain the PSAMP monitoring efforts that provide a strong foundation of historical data and knowledge throughout Puget Sound, and will improve and expand the current monitoring framework in several ways:

- Incorporate a broader ecological perspective into toxics monitoring, by expanding the suite of indicator species so it will be possible to examine the movement and biological effects of toxicants through Puget Sound food webs.

- Include new chemicals of concern that are emerging as potential threats to human and ecological health.

- Incorporate a wider range of biological measurements into the sampling program, particularly physiological and population-related endpoints that can be used for modeling and risk assessment efforts.

- Provide data for toxics-related indicators and benchmarks that can be used by managers to assess the health of Puget Sound.

- Include dedicated funding for diagnostic research studies that would help us to understand the mechanisms through which toxic chemicals are damaging Puget Sound biota, and determine exposure levels that are associated with harm.

- Provide support for localized effectiveness monitoring studies to gauge how well our toxics reduction efforts are working.

We recommend that TBiOS be integrated with other biological, physical, and chemical monitoring activities that are being conducted within the Puget Sound region. The impacts of these toxicants on the health of Puget Sound biota and their significance within the context of other natural and anthropogenic impacts cannot be fully understood without complementary information on the biology of the organisms we are studying, the physical environment they inhabit, and the sources of contaminants they may be exposed to. It will be critical to develop strong linkages between TBiOS and other monitoring programs, including: 1) contaminant monitoring in sediments and the water column; 2) physical habitat and vegetation monitoring; 3) conventional water quality monitoring (i.e., for nutrients, suspended solids, fecal coliform bacteria, temperature, salinity, dissolved oxygen, turbidity, and ambient light conditions); 4) monitoring for marine biotoxins and pathogens; and 5) population surveys of fish, shellfish, birds, and marine mammals. This information will be complementary to data collected by TBiOS, and pertinent to our larger mission of understanding how anthropogenic activities affect Puget Sound and how damage from such activities can be minimized.

Implementation of TBiOS

This concept paper provides a general description of TBiOS, its relationship to current and future monitoring in Puget Sound, and its relevance to the management and assessment questions raised by the PSP and related organizations. From this general framework, we envision that a more detailed implementation plan will be developed, with more specific suggestions on target species, endpoints, monitoring strategies, and protocols. The implementation plan will be developed through consultation with partner agencies and individuals involved in toxics and biological monitoring in the Puget Sound region, and will rely on their expertise to select the most appropriate target species, biological endpoints, contaminants, and monitoring strategies to incorporate into the TBiOS. This type of collaboration will also facilitate the development of an interagency network for carrying out the program. Those developing the monitoring plan for TBiOS will also work closely with the Puget Sound Environmental Indicators workgroup (Indicators Workgroup) to ensure that the components selected for the monitoring program are consistent with recommended water quality and related indicators, and will provide managers with the information they need to assess the health of the Sound.

Additionally, in order to implement the TBiOS, short-term studies are needed to collect baseline data on potential new indicator species, to develop analytical methods, and to ensure comparability of contaminant analyses among the various agencies and laboratories that will be involved in long-term monitoring. It will also be important to provide adequate funding for existing monitoring activities so they can continue until the TBiOS is implemented. To fill these needs we propose the following set of Puget Sound toxics monitoring and methods development studies: 1) Synoptic sampling of potential new indicator species, representing important food web components that have rarely been sampled in the past (e.g., primary producers, predators of current indicator species, and pathogens and parasites of current indicator species; 2) Continued monitoring of current indicator species (e.g., blue mussels, English sole, coho salmon, osprey, and harbor seals), with particular emphasis on non-sampled areas and historically sampled sites where temporal information is sparse. These data will help in selecting sampling stations for TBiOS; 3) Inter-laboratory comparisons of chemical analyses, to ensure that contaminant data collected as part of TBiOS by multiple agencies and laboratories are comparable; and 4) Method development for emerging contaminants, such as pharmaceuticals and wastewater compounds. Ideally, these studies would be carried out over an 18-month timeframe, and the results would be used to refine the TBiOS sampling and analysis plan. With the agreement of the PSP and participating agencies, the TBiOS could be implemented upon completion of these studies.

Conclusion

TBiOS will serve six primary functions:

1. The TBiOS will identify toxics-associated injury to the Puget Sound ecosystem, and provide information on the geographic range, extent, and severity of the problem.

2. The TBiOS will expand and refine our understanding of how toxics move through the Puget Sound ecosystem and affect wildlife and humans.

3. The TBiOS will guide our toxics reduction strategy efforts by helping to identify those watersheds where contaminants are the greatest problem, and where detailed evaluations of loadings, diagnostic studies, toxics reduction activities, and monitoring are most needed.

4. By tracking spatial and temporal trends for toxics, as well as trends in biological indicators that measure contaminant effects, TBiOS will provide a basis for evaluating the effectiveness of toxics reductions strategies as they are implemented throughout the region. It will also provide for localized effectiveness monitoring as needed.

5. The diagnostic studies will establish cause-and-effect linkages between toxicant exposure and biological impacts that will allow us to predict pollution effects, and serve as a basis for further management actions.

6. Data collected through this program on toxics concentrations and effects in biota data may be used to help develop and establish more protective water quality and sediment guidelines.

With this comprehensive framework, the TBiOS will meet the criteria laid out by the Puget Sound Partnership (the Partnership) for an improved program of toxics monitoring and reduction, as the proposed monitoring system will be 1) an "improved survey of toxic contaminants and their effects" on both Puget Sound biota and humans; 2) an integrated, comprehensive, multi-agency program; and 3) a program of investigation to characterize conditions and stressors (through regional monitoring) and test hypotheses (through diagnostic studies). By collecting data on concentrations and effects of toxic contaminants in the Puget Sound ecosystem in a consistent and strategic manner, the TBiOS will significantly enhance our ability to protect estuarine ecosystems.

Introduction

The Puget Sound Partnership has identified the ongoing input of pollutants as one of the most immediate and pervasive threats facing the Sound (PSP 2008). The Washington State statute that established the Partnership declares that a significant reduction of toxics that enter the Puget Sound's fresh and marine waters is necessary to achieve the recovery of Puget Sound (Engrossed Substitute Senate Bill 5372; PSP 2007). Preventing water pollution at its source and controlling and managing stormwater are seen as especially important priorities in the Partnership's Action Agenda for the Recovery of Puget Sound (PSP 2008). While toxics reduction is a major goal of the Partnership, the importance of monitoring as a way of measuring progress toward this goal is also strongly emphasized. During 2008, the Partnership worked with a broad group of regional scientists to identify a set of measurable indicators that can track the success of cleanup activities in the Sound (O'Neill et al. 2008). Toxics loadings and concentrations of toxics in water and sediments are recognized as important measures of pollutant stress in the Sound. However, it will be equally important to have good information on the uptake and effects of toxic contaminants in Puget Sound biota.

Why is Biological Monitoring Important?

As Karr (2006) states, the historical record shows that "managing narrowly for clean water or for some conception of "optimal habitat" has neither halted degradation nor recovered damaged water resources." This is in part because the Clean Water Act (CWA) emphasizes standards for effluents rather than biological effects and contaminant concentrations in animals residing in the receiving waters (Karr 1991). This may underestimate risk because contaminants that are barely detectable in the water column may bioconcentrate and magnify through the food chain to dangerous levels in top predators. Another problem with the standard monitoring framework is that the biological data used in setting water and sediment quality standards are not always relevant to ecological effects. The emphasis has traditionally been on the short-term effects of chemical pollutants on laboratory organisms that are exposed to the contaminant through a single source (e.g., water). In many cases, lethality is used as an endpoint, so the tests do not take into consideration the many sublethal effects that may later affect the survival and reproduction of aquatic organisms. In the environment, most animals are subject to long-term, chronic exposure from multiple sources (water, prey, and sediments). The test organisms used in toxicity bioassays typically have short life cycles and are easy to rear in the laboratory, but may not be representative of longer-lived or more sensitive species in the wild. Moreover, the responses of organisms to single contaminants may not be representative of responses in the wild, where they are likely exposed to a mixture of diverse substances that may act synergistically rather than additively, and are also subjected to additional non-chemical stressors that can exacerbate the impacts of toxicants (Laetz et al. 2009). Even when the effects of toxicants are examined in free-living animals, some impacts may be overlooked by a narrow focus on a single target species. Toxic chemicals do not always act directly, but may harm organisms by affecting their prey base or their susceptibility to pathogens (Arkoosh and Collier 2002). Subtle behavioral impacts of toxicants may reduce the animal's ability to find food, avoid predators, and engage in normal mating behavior. It is difficult to test for such effects in traditional laboratory bioassays, and they may be overlooked in field monitoring unless special studies are designed to investigate then. As the limitations of abiotic monitoring are recognized, more agencies, both nationally and internationally, are incorporating biological monitoring and assessment into their water quality programs (ANZEEC 2000; Tamai 2000; European Commission 2000).

Research shows that monitoring contaminants in sediment and water alone is not enough in Puget Sound

In Washington State, we protect our aquatic environment from the impacts of toxic contaminants largely through legislation that focuses on maintaining water and sediment quality. State mandates include Washington State's sediment management standards, which establish acceptable concentrations of a range of contaminants in marine sediments. Fish tissue residue standards are also in place for the protection of human health, and in some cases for the protection of fish and wildlife, but environmental monitoring tends to be concentrated on water and sediments. Measuring concentrations of contaminants in regional sediments and waters to determine their compliance with these standards is an important and necessary part of the effort to control and reduce toxic inputs into Puget Sound. However, research is showing us that measuring levels of contaminants in the physical environment is not enough (Gobas and Wilkerson 2003; Redman 2007).

For example, it is becoming increasingly clear that sediment contaminant levels alone may not provide a complete picture of the impacts of toxicants on the Puget Sound ecosystem. Recent surveys of sediment contamination in the Sound, conducted as part of the PSAMP, suggest that the proportion of Puget Sound sediment that is contaminated, based on Washington State Management Standards, is relatively small (Long et al. 2005). Indeed, Long et al. state, *"Relative to many other estuaries and marine bays of the USA, Puget Sound sediments ranked among those with minimal evidence of toxicant-induced degradation."* This suggests that, with the exception of animals residing in urban areas, persistent contaminants are not a major problem for aquatic life in Puget Sound.

However, the biota of Puget Sound tell a very different story. Elevated tissue contaminant levels (West et al. 2001) and liver cancer (Myers et al. 2003) in bottom fish from urban bays in the Sound have been documented by NOAA and WDFW in PSAMP monitoring, and these impacts are strongly correlated with concentrations of contaminants in sediments. But in addition to these findings, monitoring has uncovered other, unexpected impacts of toxics on Puget Sound biota, impacts that were not predicted from abiotic measures. These unexpected impacts explained in more detail below include: contamination of the pelagic food web, emerging chemicals of concern, storm water effects on Puget Sound biota, and adverse effects of chemicals in biota at levels considered healthy under sediment management standards

Contamination of the pelagic food web. Pacific herring are one of Puget Sound's keystone forage fish species. These fish spend almost all of their lives in pelagic waters, removed from direct contact with sediments and benthic prey, so should be among the least contaminated of fish species. Surprisingly however, PSAMP monitoring has shown that herring from the central and southern basins of Puget Sound have higher body burdens of persistent bioaccumulative toxics (PBTs) than herring from the severely contaminated Baltic Sea (West et al. 2008). Thus, the pelagic food web of Puget Sound appears to be more seriously contaminated than previously anticipated (West et al. 2008). National contaminant monitoring programs such as NOAA's Mussel Watch also show higher levels of PBTs in mussels suspended in the water column in Puget Sound than in many other US estuaries (PSAT 2007a). Other components of the pelagic foodweb also seem to be affected. For example, juvenile Chinook salmon from urban estuaries in Puget Sound are accumulating PBTs as concentrations above thresholds for effect on immune function, growth, and metabolism (Stein et al. 1995, Stehr et al. 2000, Arkoosh et al. 2001, Meador et al. 2002). Chinook salmon that are resident in Puget Sound (a result of natural migration patterns as well as hatchery practices) are several times more contaminated with PBTs than more migratory Puget Sound salmon and other salmon populations along the West Coast (O'Neill et al. 2007, O'Neill and West 2009). Due to associated human health concerns, fish consumption restrictions for Puget Sound salmon have been recommended by the Washington State Department of Health. Extremely high levels of chemical contaminants are also found in Puget Sound's top predators, including harbor seals and southern resident killer whales (Krahn et al. 2007, 2009; Ross et al. 2000, 2004; Ross 2006)

Although Puget Sound sediments are not as highly contaminated as those in some other urbanized estuaries of the United States, our pelagic food web may be more contaminated due to physical characteristics of Puget Sound or unique attributes of the food chain or eutrophication status. The hydrologic isolation of Puget Sound puts the Puget Sound ecosystem at higher risk for exposure to toxic chemicals that enter Puget Sound and have longer residence times within the system. This entrainment of toxics can result in biota being exposed to increased levels of contaminants for a given input, compared to other large estuaries. The problems in Puget Sound associated with contaminants are exacerbated by the added problem of biological isolation. Because Puget Sound is a deep, almost oceanic, habitat, the tendency of a number of species to migrate outside of Puget Sound is limited relative to similar species in other large urban estuaries. This high degree of residency for many marine species can result in a more protracted exposure to contaminants. It is this combination of hydrologic and biologic isolation that makes the Puget Sound ecosystem highly susceptible to inputs of toxic chemicals compared to other major estuarine ecosystems (Collier et al. 2007). High exposure of predators in Puget Sound may also result if the Puget Sound food web has large number of trophic links that increase the potential for biomagnifications.

Emerging chemicals of concern, often not detected in water or sediments, in Puget Sound biota. Various chemicals of emerging concern, such as pharmaceuticals and wastewater compounds that have rarely been monitored in water and sediments, have been observed in Puget Sound biota. Notable examples are the flame retardants, polybrominated diphenyl ethers or PBDEs, that have been identified at relatively high concentrations in some populations of marine mammals, marine fish, and salmon from Puget Sound (Ylitalo et al. 2007; O'Neill et al 2007; PSAT 2007a; Sloan et al. 2009), although concentrations in bed sediments are relatively low (Dutch and Aasen 2007; Dutch and Weakland 2009).

Juvenile salmon and benthic flatfish are also showing evidence of exposure to estrogenic compounds, based on presence of the yolk protein, vitellogenin, in males and juveniles. Moreover, in flatfish, abnormal vitellogenin production appears to be linked with alterations in reproductive timing in both male and female fish (Johnson et al. 2008). The compounds that might be responsible for inducing vitellogenin have not yet been identified, and the likely suspects are only beginning to be measured in water and sediments in Puget Sound (King County 2007).

Storm water effects on Puget Sound biota. Mixtures of contaminants in storm water may be having unexpected impacts on biota, as illustrated by pre-spawn mortality (PSM) of salmon in urban streams (McCarthy et al. 2008; Scholz et al. submitted). Beginning in the late 1990s, several agencies in the greater Seattle area began conducting fall spawner surveys to evaluate the effectiveness of local salmon habitat restoration efforts. These surveys detected a surprisingly high degree of mortality among migratory coho salmon females that were still ocean bright and had not yet spawned. In addition, adult coho from several different streams showed a similar progression of symptoms (disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that eventually led to the death of the affected animals. PSM has been observed in many lowland urban streams that have been surveyed to date, with overall levels that generally range from ~ 20% to 90% of the fall coho runs. By comparison, in non-urban (e.g., forested) drainages PSM is rare. The precise cause of PSM is not known. However, at present, the weight of evidence suggests that the widespread coho die-offs are a consequence of non-point source water pollution. It appears that coho, which enter small urban streams following fall storm events, are acutely sensitive to non-point source stormwater runoff containing complex mixtures of pollutants that typically originate from urban and residential land use activities. This phenomenon would not have been predicted from measurement of individual pollutants alone, as concentrations of individual chemicals measured do not exceed water quality guidelines.

Adverse effects on biota at contaminant concentrations considered safe under sediment management standards. Sediment management standards for Washington State water, which were developed from effects of toxicants on small, benthic invertebrates, are not always protective of fish and wildlife. For example, at present, the sediment quality goal, or "no effects level" for total PAHs, under Washington State's Sediment Quality guidelines, is 14,300 ppb dry wt (for sediment with 1% total organic carbon). However, Johnson et al. (2002) showed that at concentrations well below this level (< 10,000 ppb), bottomfish commonly exhibit liver disease, reproductive impairment, and altered growth, and the threshold level for such effects is ~2,000 ppb dry wt. Moreover, spawned herring eggs at some Puget Sound sites can accumulate concentrations of PAHs well above threshold levels associated with egg and larval toxicity, in areas where sediment PAH levels are considered acceptable (PSAT 2007a). Based on work with herring exposed to weathered crude oil, Carls et al. (1999) identified a larval mortality threshold of 22 ppb wet weight total PAH in eggs, and at this level of PAH uptake, larvae showed significant sublethal effects, such as yolk sac edema and premature hatching. Concentrations of total PAHs as high as 130 ppb wet weight are reported for Puget Sound herring eggs (PSAT 2007a).

Biological monitoring is relevant to the Puget Sound Partnership's goal of reducing toxics in Puget Sound

The Washington State statute that established the Puget Sound Partnership provides a mandate for improved toxics monitoring and toxics reduction in the Sound (Engrossed Substitute Senate Bill 5372; PSP 2007). Biological monitoring is one of our most effective tools for ensuring that we reach the Partnership's goal cleaning up Puget Sound by 2020, for several reasons:

The biota are our most effective indicators that toxics are harming the Puget Sound ecosystem. As mentioned above, a number of toxicant-related problems in the Sound were identified primarily by studying the biota. These include contamination of the pelagic foodweb, exposure to and effects of endocrine-disrupting compounds, and the unanticipated impacts of mixtures of compounds in stormwater. Biological monitoring has also informed us of situations where our current regulatory framework in not sufficiently protective of species of concern.

Biological monitoring can inform our toxics reduction efforts in several important ways. As well as being our best indicators of toxicant harm, the biota are also our best indicators of whether or not our management actions (e.g., loadings reductions, land use changes) are successful. When chemical concentrations and toxic injury in aquatic organisms decline in response to our cleanup activities, we may conclude that they have been effective (Myers et al. 2008). The biota can also provide information about the location, geographic extent, and severity of toxic contamination. By examining the biota, we can tell whether a problem is associated with urban hot spots, or with broad and diffuse contamination that may be difficult to control. These types of data can then be used to prioritize our toxics reduction efforts and select the most appropriate locations for monitoring studies or management actions. For example, biological effects such as liver lesions in flatfish and pre-spawn mortality in coho salmon show strong associations with urban land use and industrial activities (Myers et al. 2003; McCarthy et al. 2008), making it possible to target specific watersheds for more detailed evaluations of loadings, toxics reduction activities, and monitoring. On the other hand, PSAMP data on mercury concentrations in Puget Sound fish indicate that similar levels of mercury are observed in animals throughout the Sound, with age being the most important factor in determining body burdens (West et al. 2001, PSAT 2007a). In this situation, monitoring and modeling loadings of mercury in specific watersheds to identify toxics reduction activities would not be such an effective strategy. The biota can also tell us when to monitor and how often to monitor, allowing us to target time points when species of concern will be present.

Biological monitoring is key to ecosystem management.

Monitoring biota allows us to evaluate the impacts of toxics – and the effectiveness of our efforts to reduce them - in an ecosystem context. Collecting data on toxicant exposure in combination with other biological data on a broad range of species can give us a better sense of how contaminants may affect the resilience of the system. We can better understand the potential for recovery of specific species and important ecosystem functions, and improve evaluation of the impacts of toxics coupled with other stressors, as well as their potential indirect effects through the food web. Clearly, biological monitoring is needed both to direct and to evaluate the effectiveness of toxic reduction efforts on the Puget Sound ecosystem.

Biological Monitoring in Puget Sound: Current Status

Fortunately, we already have a strong tradition of biological monitoring in Puget Sound, through the Puget Sound Assessment and Monitoring Program (PSAMP). As part of this program, concentrations of contaminants and their effects are being monitoring in several species. Ongoing efforts include:

- **Contaminants in fish tissue.** WDFW, in collaboration with NOAA Fisheries, monitors contaminant concentrations in five fish species representing a wide range of habitat preferences, feeding strategies, and trophic position: English sole (benthic); quillback and copper rockfish (demersal); coho salmon (pelagic); and Pacific herring (pelagic). Focus or pilot status surveys have been conducted on a number of other species including Dungeness crab, spot prawn, ling cod and Chinook, chum, pink, and sockeye salmon.

- **Liver disease in English sole.** WDFW and NOAA evaluate PAH-associated liver disease and other pathological conditions such as gonadal lesions in English sole, as indicators of fish health and environmental quality.

- **Contaminants in harbor seals**. WDFW, NOAA, and other agencies monitor contaminant concentrations in harbor seals from Puget Sound and the Georgia Basin.

- **Benthic community structure and invertebrate toxicity**. The Washington State Department of Ecology (Ecology) monitors marine sediment toxicity with invertebrate bioassays (e.g., amphipod survival, sea urchin fertilization, Microtox, and enchinoderm embryo development), and evaluates benthic community structure at sites throughout Puget Sound where sediment contaminants are measured (Long et al. 2005). Ecology conducts a temporal monitoring program to monitor long-term trends in sediment toxicity at a set of fixed stations throughout the Sound, and a spatial monitoring program that does more intensive sampling within eight monitoring regions of the Sound (i.e., the Strait of Georgia, San Juan Archipelago, Eastern Strait of Juan de Fuca, Admiralty Inlet, Whidbey Basin, Central Sound, Hood Canal, and South Sound). One region is sampled intensively each year, on a rotational cycle (Dutch et al. 2009).

- **USGS bird contaminant studies**. The USGS has studied the measured concentrations of dioxins, furans, PCBs, and PBDEs in osprey eggs in Puget Sound, and examined the effects of these contaminants on the nesting success of Puget Sound osprey populations (PSAT 2007a). Ospreys are fish-eating birds at the top of the food web, and they capture fish within a relatively short distance from their nest sites so are representative of local conditions. USGS scientists have collected and analyzed eggs from nesting osprey near Everett Harbor, the Duwamish River, and Lake Washington between 2002 and 2004 (PSAT 2007a). In 2006, contaminant exposure and effects on osprey nesting along the Lower Duwamish Waterway in Seattle were assessed as part of the Natural Resource Damage Assessment in cooperation with the U.S. Fish and Wildlife Service. In another study, several current-use herbicides were measured in the eggs of osprey from 15 sites from five study areas Puget Sound/Seattle area (Chu et al. 2007). The USGS also collected some information on trace elements and POPs in surf scoters in the mid-1980s (Henney et al. 1991), but no recent data are available.

- **USFWS bird contaminant studies.** The USFWS has conducted some work on contaminant levels in marine birds, including analyses of bald eagle eggs collected from Hood Canal (Mahaffy et al. 2001, PSAT 2007a) and surf scoters in Commencement Bay (Mahaffy et al. 1997) but monitoring is quite limited. In the mid-1980s, other researchers conducted studies on eggshell thinning and organochlorine contaminants in several species of Puget Sound waterbirds, including cormorants, great blue herons, pigeon guillemot, and glacous-winged gulls (Fitzner et al. 1988; Speich et al. 1992), but little follow-up work has been done in more recent years.

- **DFO/NOAA killer whale work.** NOAA and the Canadian Department of Fisheries and Oceans (DFO) have measured persistent organic pollutants, including PCBs, DDTs, and PBDEs, in biopsy samples from southern resident killer whales (*Orcinus orca*) that reside in Puget Sound, the Strait of Juan de Fuca, and the Strait of Georgia (Ross et al. 2000, 2004; Krahn et al. 2007, 2009), and used this information to investigate possible contributing factors for population declines.

- **Focus Studies.** In addition to regional status and trends monitoring, PSAMP has also conducted focus studies to address special problems within the Sound. As described earlier in this document, WDFW/NOAA focus studies conducted in conjunction with PSAMP have examined exposure to and effects of xenoestrogens in English sole and impacts of PAHs on herring embryo development in Puget Sound. Ecology also conducts focus studies to address special problems associated with sediment toxicity. Examples include a study to provide sediment quality data and information on low dissolved oxygen problems in Hood Canal (Dutch et al. 2007, Long et al. 2007) and effectiveness monitoring studies in Elliott Bay/Lower Duwamish and Commencement Bay to assess the overall extent of sediment contamination, changes in sediment quality over time, and the long-term effectiveness of collective toxics management efforts in these bays (Partridge et al. 2008).

While this is valuable work, it does not constitute a comprehensive, ecosystem-side program. The species involved are fairly limited, important trophic relationships and food-web linkages are not included, only a few biological effects are examined, and efforts carried out on different species by different agencies are not always well integrated. The sentinel species that have been monitored in Puget Sound as part of PSAMP and related programs, some of their characteristics, and the information that has been collected on them are summarized in Table 1. As the table shows, biological contaminant monitoring has been focused heavily on fish, along with few species of endangered marine mammals, such as orcas. Some of the more obvious data gaps include phytoplankton and zooplankton; benthic invertebrates, sampled either directly, or as stomach contents of higher order benthic feeders; and higher levels predators, especially birds. Also, while PSAMP has included some focus studies, or diagnostic studies that establish cause-and-effect linkages between toxicant exposure and injury to biota, support for this type of work has been limited.

The Puget Sound Action Team (now the Puget Sound Partnership) identified similar data gaps in its review of the status of toxics research and monitoring in Puget Sound (PSAT 2007a). The items mentioned included the lack of data on contaminant levels and effects in benthic infauna other than harvested large mollusks; unknown effects of reproductive toxicants and endocrine disrupting compounds in fish; limited data for assessing temporal trends in organochlorine compounds in killer whales; and a lack of data on contaminant concentrations and effects in birds.

A key item on the Puget Sound Partnerships' agenda is "to develop plans for improved surveys of toxic contaminants and their effects, including effects on human health and the biological organisms of the Puget Sound ecosystem". The Partnership further recommends that "scientists from government and elsewhere should collaborate in an integrated, comprehensive program of scientific investigation to characterize conditions and stressors, [and] test hypotheses relevant to toxics harm and control". To assist in carrying out these objectives, we recommend that biologically-based ecosystem-wide monitoring for toxics should be implemented in Puget Sound, through a program that we call a biological observing system for toxic contaminants (TBiOS).

TBios Project Description

What is TBiOS?

The TBiOS is a biologically based framework for toxics monitoring and research that assesses exposure and effects of chemical contaminants in biota, integrated across ecologically relevant habitats and food webs. The species, habitats, and life stages and effects to be monitored as part of the TBiOS will be guided in part by a conceptual model that describes the fate and transport of toxics in Puget Sound, which is being developed from previously collected PSAMP data. The model will assist in selecting suites of species that are representative of important pathways for contaminant exposure, and are linked through the food web for various interactive and indirect effects to be observed more readily.

The TBiOS will include three major components:

1. **A region-wide monitoring program** to provide information on long-term geographic and temporal trends, performance measures, early warnings, and the ability to predict fate, transport, and effects of many new pollutants.

2. **Localized effectiveness monitoring** to determine how well selected toxicant reduction actions are succeeding (e.g., monitoring after restoration, remediation, or source control).

3. **Diagnostic studies** to investigate and predict new relationships, trends, and special problems related to toxicants that are identified from the monitoring data, and to develop new indicators and analytical methods.

These specific components are described in greater detail below. As noted above, the PSAMP already includes these components, but these programs will be strengthened and expanded with TBiOS, particularly in cases when there are deficits in research and monitoring of biota.

Region-wide monitoring program

The region-wide monitoring program will maintain and expand the monitoring efforts that have been conducted under the PSAMP and within the region. Although the indicators that have been measured over the past 20 years provide a strong foundation of historical data and knowledge throughout Puget Sound, the TBiOS would:

1. Include new chemicals of concern (e.g., pharmaceuticals, personal care products, current use pesticides) that are emerging as potential threats to human and ecological health, and help to develop the ability to predict the fate, transport, and effects of these new chemicals in the system.

2. Incorporate a broader ecological perspective into toxics monitoring by examining the movement and biological effects of toxicants through Puget Sound food webs. This is an especially important issue, in view of the evidence that current monitoring is providing about the importance of the pelagic food web as a source of contaminants in the Sound.

3. Incorporate a wider range of biological measurements into the sampling program, particularly the types of measurements that would provide data on physiological and population effects in key species that can be used for modeling and risk assessment efforts.

4. Integrate with other research and monitoring efforts in Puget Sound, especially those that focus on the ecological health of the Sound, to improve our understanding of the responses of biota to multiple stressors.

5. Provide data for toxics-related indicators and benchmarks that can be used for managers to assess the health of Puget Sound. Such indicators are already used by PSAMP to characterize the health of Puget Sound (PSAT 2007b). The list of current and potential indicators is currently being reviewed by the Indicators Workgroup (O'Neill et al. 2008), and the list of relevant measurements may be improved and expanded as part of TBiOS. It might also include new composite measures, similar to the Index of Biotic Integrity (IBI), that have been useful in assessing environmental degradation in other aquatic systems (e.g., Morley and Karr 2002; Karr 2006), and will be one means of integrating effects of multiple stressors.

Contaminants to be measured

A preliminary analysis of toxic contaminant loadings in Puget Sound showed that runoff from land surfaces and air deposition directly to marine waters are the principal routes whereby toxicants enter Puget Sound (Hart Crowser Inc. et al. 2007). This study also indicated the presence of emerging chemicals of concern in Puget Sound, compounds that are only beginning to be monitored and whose effects on marine organisms are largely unknown. Table 2 provides a preliminary list of the toxics that TBiOS might investigate in Puget Sound biota in terms of exposure and effects. The selection of chemicals is based on the following ongoing efforts: 1) the Puget Sound toxics group; 2) The PSAT report that summarizes the potential effects of contaminants of concern in Puget Sound and 3) Land use-associated surface runoff loadings throughout Puget Sound (Hart Crowser Inc. et al. 2007).

Established contaminants of concern. A number of contaminants are already being monitored in Puget Sound biota, in conjunction with PSAMP. These include PCBs, PBDEs, organochlorine pesticides, including DDTs, and various metals (arsenic, copper, lead, and mercury). It is important to continue to monitor these chemicals, most likely expanding or altering the target species and tissues to better assess foodweb transfer and effects.

Emerging contaminants of concern in Puget Sound. A variety of pharmaceuticals that may cause harm to aquatic life even in small quantities have been found in fresh and marine waters throughout the world (Kolpin et al. 2002; Bound and Voulvoulis 2005). There is also increasing concern about the impact of contaminants that originate from common household products, such as cosmetics, detergents, and toiletries. These personal care products, as well as pharmaceuticals including antibiotics, birth control pills, analgesics, and chemotherapy agents, are reaching the aquatic environment via the sewage system. Antibiotics that are fed to livestock are also finding their way into the environment through manure that is used as a fertilizer and transported to the aquatic environment in runoff.

Koplin et al. (2002) showed that the most frequently detected chemicals from the 139 streams sampled across the United States (including streams in Puget Sound) were the following: coprostanol (fecal steroid), N-N-diethyltoluamide (insect repellent), caffeine (stimulant), triclosan (antimicrobial disinfectant), tri (2-chloroethyl) phosphate (fire retardant), and 4-nonylphenol (non-ionic detergent metabolite). In general, steroids, non-prescription drugs, and insect repellents were the groups of chemicals most commonly found, but detergent metabolites, plasticizers, and steroids were detected at the greatest concentrations.

Little is known about the levels, effects, transport, and ultimate fate of these emerging chemicals in Puget Sound biota. Currently there are few analytical methods to measure most of these compounds in environmental samples, particularly biota, and this is one of the reasons why the levels and effects of these compounds in biota and humans are poorly understood. Table 2 shows a list of potential effects of these compounds, some of which have not yet been documented for Puget Sound.

Biological matrices in which contaminants will be measured. The potential biological matrices in which these contaminants will be measured and the effects-endpoints to be measured in biota (Table 2) will vary depending on the chemical, as some compounds are bioaccumulative and others are not. In some cases it is easier to measure the effect (e.g. impaired olfactory function in salmon) than to measure the cause (e.g. exposure to current use pesticides) because the chemicals of concern are transient and not easily detected in biota. Recommended target tissues for bioaccumulative contaminants, such as PCBs, DDTs, and PBDEs, will include muscle (for assessment of human health risk); whole body (for correlation with health effects in the target organisms); eggs or ovaries (to evaluate risks of maternal transfer); and prey species or stomach contents (to estimate dietary uptake of contaminants). Recommended target tissues for non-bioaccumulative contaminants will include bile and blood (e.g., for PAHs, pharmaceuticals, certain environmental estrogens).

Species to be monitored

Species currently monitored for toxicant exposure as part of PSAMP and associated programs include English sole, Pacific herring, quillback and copper rockfish, coho salmon, harbor seals, killer whales, mussels, and osprey. Table 1 summarizes the types of chemical and biological data that have been collected for these and other sentinel species from known studies in the

Sound. Ideally, the TBiOS will build on the historical data generated from these studies, but will also include new species that have been neglected in other programs.

Species selected for monitoring in the TBiOS will be predominantly marine and estuarine. The choice of monitoring species will be guided in part by conceptual models of contaminant transfer in Puget Sound (e.g. Newton et al. 2000), so that the suites of species chosen are representative of important routes of contaminant exposure, and are linked through the food web so various interactive and indirect effects can be observed more readily. For example, a suite of organisms representative of the pelagic food web might include phytoplankton and zooplankton, herring, Chinook or coho salmon, and orca whales. A suite of organisms representative of the benthic food web might include clams and polychaete worms, English sole, lingcod, and harbor seals (Table 1). Species of high economic and ecological importance will also be a priority. Table 1 presents a list of potential sentinel species for the TBiOS, along with characteristics that may be used to rank their suitability for monitoring. Some important considerations include the species range, its catchability, its longevity, site fidelity and residency, habitat preference, feeding strategy, and whether or not historical data are available for the species for assessing long-term trends of contaminant concentrations.

Biological endpoints to be monitored

One of the most important components of the TBiOS will be measurement of biological characteristics that can be predictive of contaminants exposure and effects. Currently, PSAMP measures fish length, weight, age, and liver and gonadal disease. Liver disease is highly correlated with exposure to carcinogenic PAHs, and is a very effective way of monitoring exposure to and effects of this particular class of chemicals in Puget Sound fish. Gonadal lesions are good indicators of reproductive impairment, but can be caused by a wide range of chemicals, including PAHs, PCBs, DDTs, and certain metals, as well as synthetic estrogens. Biological indicators under TBiOS will be expanded to include a wider range of predictive covariate physiological effects measures, including:

- **Lipid content and classes –** a good measure of nutritional condition, and also needed for comparison of concentrations of bioaccumulative contaminants.

- **Somatic indices (condition index, liver somatic index***)* **–** easily measured; condition index is a good indicator of nutritional status, and high liver somatic index is associated with contaminant exposure.

- **Plasma vitellogenin –** in male and juveniles, an indicator of exposure to estrogenic compounds; can also be useful as an indicator of reproductive condition in maturing females.

- **Histopathology (liver, gonad, spleen) –** important way to identify toxicopathic lesions, including cancer, gonad abnormalities, and other abnormalities.

- **Stomach contents taxonomy –** important for understanding sources of contaminants in the diet and understanding contaminant transfer through food webs.

- **Otolith analyses –** provides data on the age of the animal, which can be important in interpreting other biological indicators; also provides data on instantaneous growth rates.

- **Biochemical indicators** of growth and metabolism (e.g., insulin-like growth factor, thyroid hormones), as well as reproductive status (e.g., reproductive hormones) where appropriate.

Localized effectiveness monitoring

Studies conducted under this component of the program will monitor the effectiveness of regional and local efforts to reduce toxics in the Sound (e.g., remediation and restoration activities, source control, or changes in wastewater treatment) to determine how well specific toxicant reduction actions were succeeding. They differ from the region-wide monitoring efforts in that they will focus on specific cleanup or source control projects and will be limited in geographic area and temporal extent. These studies will be designed to complement, and will be coordinated with any remediation and restoration monitoring that might be mandated as part of Natural Resource Damage Assessment and Restoration (NRDA), Superfund, or related regulatory actions. Ecology's PSAMP Sediment Team is currently conducting effectiveness monitoring for toxics in sediments at the bay-scale through Ecology's Urban Waters Initiative (Partridge et al. 2009). With TBiOS, complementary biological monitoring will be added to existing PSAMP efforts.

The effectiveness monitoring projects will apply a set of specific indicators appropriate for the contaminant and effects of concern. For example, at a site near a sewage outfall where new wastewater treatment methods had been applied, male flatfish might be monitored for vitellogenin induction, an indicator of exposure to environmental estrogens that frequently appear to enter the aquatic environment through wastewater discharges. At a site where sediment remediation had occurred because of PAH contamination, the suite of indicators might include PAH concentrations in Dungeness crab hepatopancreas, liver disease in flatfish, PAH metabolite levels in fish bile, and PAH-associated DNA damage in the liver, all of which are effects that are linked with PAH exposure through established cause- and-effect relationships (Myers et al. 2003).

An example of an effectiveness monitoring study that has been carried out partially under the existing PSAMP program is post-capping monitoring of PAH contaminated sediments in Eagle Harbor (Myers et al., 2008). Eagle Harbor in Puget Sound became a Superfund site in 1987 due to high sediment concentrations of PAHs released chronically from a nearby creosoting facility. Prior to site remediation, hepatic lesion prevalences and biomarker values in English sole from Eagle Harbor were among the highest in Puget Sound. In 1993 and 1994, a primary cap of clean sediment was placed over 54 acres of the most contaminated portions of Eagle Harbor, with a secondary cap added between November 2000 and February 2002, to sequester PAH-contaminated sediments. Lesion prevalences and biomarker values just before capping and 3 years after capping were reduced compared to historical data, consistent with closure of the creosoting facility closure in 1988 and the capping process. These results show that the sediment capping process has been extremely effective in ameliorating PAH exposure and associated biological effects in resident flatfish species. They also demonstrated that longer term monitoring of pollutant responses in biological resources, such as resident fish, can show the efficacy of this type of contaminant remediation. Under the TBiOS, similar studies will be incorporated into regional sampling as appropriate.

Diagnostic Studies

Diagnostic studies will investigate special problems related to toxicants in the Sound. These studies may include both field sampling and targeted laboratory research, and will address issues such as development of new indicators and analytical methods that can be incorporated into the long-term monitoring program, establishing cause-and-effect relationships between toxicant exposure and biological alterations seen in field studies, and determining threshold effect concentrations for species and contaminants of concern, for eventual incorporation into the regulatory framework. The diagnostic studies might also collect data on specific issues or problems related to toxic contaminants, e.g., studies on growth, reproduction, disease resistance, and behavior of species of concern. These are studies that cannot be easily incorporated into the regional monitoring program because of special sampling needs. For example, field studies on the reproductive and developmental effects of contaminants, which can only be carried out during the mating season, may be performed as diagnostic studies. Studies that require a laboratory setting to measure endpoints of interest, such as studies on the behavioral impacts of toxicants, or disease challenge studies to test for immune dysfunction, are other examples of possible diagnostic studies. Recent PSAMP diagnostic studies that have yielded especially valuable information about the health of Puget Sound biota include a joint WDFW/NOAA investigation of the effects of toxicants on Pacific herring (Incardona et al 2009), and a WDFW/NOAA the investigation of xenoestrogen exposure and effects in English sole (Johnson et al. 2008).

Objectives of TBiOS – A Summary

Briefly, the TBiOS will serve six primary functions:

1. The TBiOS will identify toxics-associated injury to the Puget Sound ecosystem, and provide information on the geographic range, extent, and severity of the problem.

2. The TBiOS will expand and refine our understanding of how toxics move through the Puget Sound ecosystem and accumulate in wildlife and, ultimately, through fish and shellfish consumption, in humans.

3. The TBiOS will guide our toxics reduction strategy efforts by helping to identify those watersheds where contaminants are the greatest problem, and where detailed evaluations of loadings, diagnostic studies, toxics reduction activities, and monitoring are most needed.

4. By tracking spatial and temporal trends for toxics, as well as trends in biological indicators that measure contaminant effects, TBiOS will provide a basis for evaluating the effectiveness of toxics reductions strategies as they are implemented throughout the region. It will also provide for localized effectiveness monitoring as needed.

5. The diagnostic studies will establish cause-and-effect linkages between toxicant exposure and biological impacts that will allow us to predict pollution-effects, and serve as a basis for further management actions.

6. Data collected through this program on toxics concentrations and effects in biota data may be used to help develop and establish more protective water quality and sediment guideline

INTEGRATION OF TBIOS WITH OTHER PUGET SOUND PROGRAMS

TBiOS and Other Monitoring Programs

The TBiOS described herein is focused on monitoring toxics exposure and effects in Puget Sound biota, but, as noted earlier, exposure to toxicants occurs within an ecosystem context. The impacts of toxicants on the health of Puget Sound biota and their significance within the context of other natural and anthropogenic impacts cannot be fully understood without complementary information on the biology of the organisms we are studying, the physical environment they inhabit, and the sources of contaminants they may be exposed to. Thus the TBiOS should be integrated with other types of biological, physical, and chemical monitoring that are taking place within the Puget Sound region. Current monitoring programs and activities that are relevant to TBiOS include:

1. **Abiotic contaminant monitoring.** The Washington State Department of Ecology currently collects information on contaminants in sediment at stations throughout Puget Sound. The King County Department of Natural Resources measures contaminants in sediments at additional stations within the greater Seattle area. King County and USGS are monitoring concentrations of selected toxic contaminants in the water column at stations in the greater Seattle area.

2. **Physical habitat and vegetation monitoring.** The Washington State Department of Natural Resources maps physical shoreline attributes throughout Puget Sound, which are important indicators of changes in land use, and related alterations in the nearshore environment, such as shoreline armoring. Washington Department of Natural Resources also monitors the presence and abundance of eelgrass and other aquatic macrophytes along the shoreline of Puget Sound.

3. **Conventional water quality**. Washington State Department of Ecology currently collects information on nutrients, suspended solids, fecal coliform bacteria, metals, temperature, and dissolved oxygen at 30 freshwater stations in the Puget Sound drainage basin, and collects data on salinity, temperature, dissolved oxygen, turbidity, ambient light conditions, fecal coliform bacteria, and nutrients at 30 marine stations within Puget Sound. King County is monitoring nutrients, pathogens, and related parameters in the water column at additional stations in the greater Seattle area. The Washington State Department of Health also monitors fecal coliform concentrations in seawater at shellfish growing areas around Puget Sound several times per year, and conducts marine biotoxin surveys to measure paralytic shellfish poison and other biotoxins.

4. **Population surveys of fish, shellfish, birds, and marine mammals.** These surveys provide important data on the abundance of key species in Puget Sound, which may be affected by toxicant exposure and other types of human disturbance. Ecology monitors benthic community structure at marine sediment sites throughout Puget Sound, while WDFW conducts bottom trawls to estimate groundfish abundance in Puget Sound. WDFW also monitors the number and condition of harbor seals at Gertrude Island in south Puget Sound, and takes part in annual aerial assessments of harbor seal numbers throughout Puget Sound. WDFW conducts aerial surveys of the population size of marine diving ducks and other diving birds in Puget Sound, and WDFW and USFWS conduct surveys of pigeon guillemot colonies to estimate the number of year-round residents.

All this information will be complementary to data collected by TBiOS, and pertinent to our larger mission of understanding how anthropogenic activities affect Puget Sound and how damage from such activities can be minimized. Currently these monitoring and research efforts are conducted by separate agencies with somewhat different goals. While strong linkages exist between some programs within PSAMP, such as the sediment toxics monitoring component conducted by Ecology, and the fish toxics monitoring component conducted by WDFW, in many cases there is only limited coordination among programs. The challenge for Puget Sound researchers will be to develop a multidisciplinary and multi-agency sampling strategy that will maximize our ability to integrate these various types of data with toxicology data collected as part of TBiOS. It will be important for researchers to meet this challenge, because the most powerful associations can be made when multidisciplinary data are collected simultaneously.

TBIOS and the Puget Sound Action Agenda

The Washington State statute that established the Partnership declares that a significant reduction of toxics that enter the Puget Sound’s fresh and marine waters is necessary to achieve the recovery of Puget Sound (Engrossed Substitute Senate Bill 5372). The Partnership proposes to do this through an interagency strategy that will include 1) the identification of long-term, numeric or quantifiable targets for toxics reduction that describe the desired condition by 2020 of key components of the ecosystem, and the establishment of benchmark interim milestones that indicate progress towards measurable outcomes; 2) the development of environmental indicators to characterize and communicate the condition of the ecosystem with respect to toxic contaminants; and 3) the collection of biological data to assess progress toward the benchmarks and provide data to calculate values for the indicators. The Partnership established an Indicators Technical Working Group to develop a set of environmental indicators for Puget Sound. This group has identified a set of recommended available environmental indicators for water quality (Table 3; O’Neill et al. 2008), and will be involved in defining a monitoring program to support indicator reporting and other information needs (O’Neill et al. 2008).

An additional part of the Partnership’s agenda is “to develop plans for improved surveys of toxic contaminants and their effects, including effects on human health and the biological organisms of the Puget Sound ecosystem”. The Partnership further recommends “that scientists from government and elsewhere should collaborate in an integrated, comprehensive program of scientific investigation to characterize conditions and stressors, and test hypotheses relevant to toxics harm and control (e.g. exposure to chemical X disrupts hormone function in flatfish, concentrations of chemical Y are constant over time)". The TBiOS is designed to meet the criteria, as the proposed monitoring system will be 1) an "improved survey of toxic contaminants and their effects" on Puget Sound biota; 2) an integrated, comprehensive, multi-agency program; and 3) a program of investigation to characterize conditions and stressors (through regional monitoring) and test hypotheses (through diagnostic studies).

In addition to the broad program criteria laid out above by the Partnership, current monitoring efforts such as PSAMP have identified key uncertainties related to toxics in Puget Sound that must be investigated to fulfill the Partnership's goal of reducing toxicant impacts in Puget Sound. The uncertainties relating to exposure and effects in biota include but are not limited to the following:

1. Relative contributions of contaminant exposures to key organisms from various loading pathways and sources (including exposure outside Puget Sound, e.g., to salmon and orcas).

2. The extent of the major reservoirs of toxics in the biota within the Sound's ecosystem.

3. The pathways of exposure and effects of emerging contaminants of concern, such as endocrine disruptors and PBDEs.

4. The effects of exposures to mixtures of toxics, and of toxic chemicals in combination with other stresses to the ecosystem (such as climate change, pathogens, and altered food supply). These multi-factor issues are likely contributing to some recently observed biological harm (e.g., pre-spawn mortality of coho salmon, and reproductive disruption in English sole).

5. Amount of toxics reduction needed to attain safe levels in the environment for humans and biota, and how this may be affected by the different life history strategies of the target species of concern. For example, reduction of persistent organic pollutant (POP) concentrations in long-lived species will be much slower than those measured in species with shorter life spans. Such concerns should be kept in mind when determining 'safe levels' for each species.

6. The effectiveness of current and potential toxics reduction measures in reducing contaminant loads in Puget Sound biota.

7. Timeframe for and extent of recovery once corrective actions (cleanups or reductions in loading) are implemented.

The TBiOS directly addresses these data needs by collecting data on concentrations and effects of toxic contaminants in the Puget Sound ecosystem in a consistent and strategic manner. It will also provide data on movement of toxic contaminants through the Puget Sound food web and provide the kind of information that will be needed for risk modeling and assessment and for evaluating long-term effectiveness of management actions in the Sound.

TBiOS and the Development of Environmental Indicators for Puget Sound

As mentioned above, Partnership has developed an Action Agenda to serve as a roadmap for restoring and maintaining the health of Puget Sound. To evaluate progress towards meeting its goals, the Partnership is also developing a set of measurable environmental indicators that will inform policy makers about environmental problems, identify key stressors impacting the environment, and assess the effectiveness of policy responses to these problems (O'Neill et al. 2008). An Indicators Technical Working Group, made up of over 40 individuals with a scientific expertise in a wide range of environmental fields, was formed to undertake this task.

The Indicators Technical Working Group has identified a set of recommended available environmental indicators for water quality (Table 3; O'Neill et al. 2008). This set of indicators includes a several endpoints that are already part of PSAMP and may be included in TBiOS (e.g., marine benthic infaunal community structure, concentrations of toxic contaminants in benthic and pelagic marine fish, and liver disease in English sole), as well as other available indicators that appear promising but have been monitored less consistently (e.g., concentrations of toxic contaminants in clams and mussels, juvenile salmon, osprey eggs, and harbor seals, as well as some indices such as the sediment quality triad and a fish tissue contaminants index). The Indicators Workgroup also suggested some potential future indicators (toxics in crab and shrimp, bioaccumulation monitoring, pesticide poisoning in raptors, toxics contaminants in herring eggs, toxic contaminants in cormorant eggs, vitellogenin induction in male fish, StAR protein and DNA damage) that might be useful as part of a monitoring and evaluation program. Finally, various data gaps were identified. Those most relevant to TBiOS include concentrations of toxic contaminants in plankton and geoducks; effects of contaminants on species population, community, and community structure; effects of stormwater pollutants and contaminants of emerging concern in ecological communities; toxics-related declines in amphibians.

During the next phase of the indicator development process, the Indicators Workgroup will generate the final selection of currently available indicators, select a suite of indicators for the Partnership, and refine and develop monitoring programs to support indicator reporting and other information needs (O'Neill et al. 2008). The development of an implementation plan for TBiOS, will be linked closely to this process, to ensure that biological endpoints selected for the monitoring plan and those recommended as indicators are consistent and will supply relevant information to managers.

NEXT STEPS IN DEVELOPMENT AND IMPLMENTATION OF TBIOS

This concept paper provides a general description of TBiOS, its relationship to current and future monitoring in Puget Sound, and its relevance to management and assessment questions raised by the Partnership and related organizations.

In order to implement the TBiOS, short-term studies are needed to collect baseline data on potential new indicator species, to develop analytical methods, and to ensure comparability of contaminant analyses among the various agencies and laboratories that will be involved in long-term monitoring. It will also be important to provide adequate funding for existing monitoring activities so they can continue until the TBiOS is implemented. To fill these needs we propose a set of Puget Sound toxics monitoring and methods development studies, to be carried out over an 18-month timeframe, that will incorporate the following components:

1. **Synoptic sampling of potential new indicator species.** This study will be a synoptic, one-time sampling of contaminant concentrations in different species across the food web to help determine the indicator species that can be used in future monitoring. The emphasis will be on species representing important food web components that have rarely been sampled in the past, including primary producers and prey and predators of current indicator species. Organisms proposed for sampling include: phytoplankton and zooplankton, benthic infauna, shrimp, crabs, small schooling pelagic fish species other than Pacific herring, pelagic fish (e.g., hake), sea birds, and marine mammals (e.g., harbor porpoise). This set of species will be sampled from a range of contaminated locations (e.g., worst case, best case and intermediate contaminated environments) to help us to better understand toxics movement in the food web.

2. **Continued monitoring of presently used indicator species.** Development and implementation of the new TBiOS monitoring program in Puget Sound may take some time, and during that process it is important to support current biological monitoring efforts. Some of these programs have always been under funded (e.g. toxics in harbor seals), while other programs (e.g., PSAMP fish contaminant monitoring) have been substantially reduced in scope in recent years. In addition, certain long-term programs (NOAA Mussel Watch) are scheduled to have their funding discontinued. The objective of this project is to provide support for geographical expansion to non-sampled areas and restoration to historical geo-spatial coverage for indicator species that are already part of our current biological monitoring programs (blue mussels, English sole, coho salmon, osprey, and harbor seals). These data will help in selecting sampling stations for TBiOS.

3. **Inter-Laboratory Comparisons for Chemical Analyses.** Measuring concentrations of contaminants in regional sediments and waters to determine their compliance with standards is an important and necessary part of the effort to control and reduce toxic inputs into Puget Sound. When TBiOS is implemented, multiple agencies and laboratories are likely to be involved in analyzing samples for contaminants in different tissue matrices and various organisms. It will be important to ensure that these data are comparable, and can be combined with existing data to document historical trends. In order to achieve this, it is necessary to first determine analytical biases across laboratories and determine the next steps needed to combine these data for trend analyses. This task contains two main components: 1) Comparison of species-specific results of different analytical methodologies among laboratories such as: Columbia Analytical (analysis for TBT); Axys Analytical Laboratories, Manchester, NOAA Fisheries, Department of Ecology and King County; and 2) Conducting cross comparisons of different matrices (e.g., muscle vs. liver, skin on and skin off fillets). The quality assurance procedures will focus on major chemical classes that are routinely measured (e.g., PAHs, PCBs, Hg) as well as important biological measurements such as lipid content.

4. **Method Development for Emerging Contaminants.** Various emerging chemicals of concern**,** such as pharmaceuticals and wastewater compounds, which as yet have been rarely monitored in water and sediments, are beginning to be observed in Puget Sound biota. One of the goals of TBiOS and the Partnership's toxics monitoring strategy is to incorporate these new contaminants of concern into regional monitoring programs. However, as yet the techniques for measuring some of these compounds, especially in biota, are not well developed. This funding will support the development of analytical techniques for measuring pharmaceuticals and wastewater compounds in biota by regional laboratories that will be involved in Puget Sound monitoring.

A second major goal is to develop a draft implementation plan that explains in more detail the scope of a TBiOS for Puget Sound region, with more specific suggestions on target species, endpoints, monitoring strategies, and protocols, which will be made available to the Partnership and representatives from partner agencies. During the process of developing the implementation plan, we anticipate that a series of workshops and/or expert interviews will be conducted with partners and individuals from different agencies involved in toxics and biological monitoring in the Puget Sound region. The objective of the workshops and interviews will be to get input from the participants on the most appropriate target species, biological endpoints, contaminants, and monitoring strategies to incorporate into the TBiOS, and to begin to develop an interagency network for carrying out the program. Those developing the implementation plan would also work closely with the Indicators Workgroup to ensure that endpoints and target species proposed for TBiOS are consistent with recommended water quality and toxics-related indicators. This information will be used to refine the draft plan. The plan will also be modified as necessary to incorporate information gathered as part of the field and laboratory studies proposed above. With the approval of the Partnership and collaborating agencies, the plan will be put into effect following completion of the background monitoring and methods development studies outlined above.

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Table 1. Matrix of potential sentinal species for TBiOS monitoring

Species	Existing Monitoring	Ubiquity	Catchability	Longevity (years)	Home Range	Habitat Preference	Logical Group	Feeding Guild or Strategy	Permit Issues	PAH Exposure	POP Exposure	Metals Exposure
Fish												
English Sole (adults)	yes	high	high	20	moderate (with homing)	benthic soft sediment	flatfish	benthic carnivore	no	extensive bile FACs	extensive muscle and liver	muscle liver
English Sole (juveniles)	yes	high	high	NA	small	nearshore soft sediment	flatfish	benthic carnivore	no	bile FACs	muscle	muscle?
Pacific Staghorn Sculpin	no	high	high	10	small	benthic nearshore can be estuarine	sculpins	benthic carnivore	no	bile FACs	muscle	muscle?
Demersal Rockfish (e.g. quillback)	1990-2000	low	high	70	small	hard substrate reefs	rockfish	demersal carnivore	yes (species of concern)	bile FACs	muscle liver	muscle liver
Ratfish (spotted ratfish)	no	high	high	unknown (hard to age)	unknown	featureless soft sediment	chimeras	benthic carnivore	no	no	some whole body	some whole body
Pacific Herring	yes	mod	moderate	7	unknown (presumed moderate)	pelagic	small, schooling pelagic planktivores	zooplanktivore	no	extensive bile FACs	whole body	whole
Herring Eggs	no	mod	high	NA	none	eelgrass, rocks	benthic embryo	NA	no	embryos	embryos	embryos
Shiner Perch/Surfperch	no	high	high	6 (most: 1-3)	presumed moderate	shallow marine estuarine	surfperches	pelagic and epibenthic picker	no	no	muscle/larvae	muscle?
Spiny Dogfish	no	moderate	moderate	80	large	pelagic and demersal	sharks	wide-ranging carnivore	maybe	no	muscle/embryos	muscle
Six Gill Shark	no	low	moderate	high (~80)	large (highly migratory)	demersal	sharks	demersal carnivore	yes	no	muscle	muscle
Coho Salmon (adults)	yes	high	high	3	large (highly migratory)	pelagic	salmon	pelagic carnivore	few	bile FACs	muscle whole body	muscle
Coho Salmon (juveniles)	no	moderate	moderate	NA	migratory	nearshore	salmon	zooplankton	few	bile FACs	whole body liver	NA
Chinook Salmon (adults)	sporadic	high	mod/low	5	large (highly migratory)	pelagic	salmon	pelagic/demersal carnivore	yes	bile FACs	muscle whole body	muscle
Chinook Salmon (juveniles)	no	moderate	moderate	NA	migratory	nearshore	salmon	pelagic/demersal carnivore	yes	bile FACs	whole body	NA
Pacific Hake	no	low	moderate	20	(moderate/unknown)	pelagic	codfish	pelagic carnivore	no	bile FACs	muscle	muscle
Invertebrates												
Dungeness Crab	no	high	high	8-10	moderate	benthic soft sediments	crabs	benthic carnivore	minor	hepatopancreas muscle	hepatopancreas muscle	muscle
Mytilus spp. (mussel)	yes (Mussel Watch)	high	high	<10 (typically)	none	shallow nearshore hard substrate	bivalves	filter feeder	no	PAH (whole body)	whole body	whole body

Table 1. Matrix of potential sentinal species for TBiOS monitoring

Species	Existing Monitoring	Ubiquity	Catchability	Longevity (years)	Home Range	Habitat Preference	Logical Group	Feeding Guild or Strategy	Permit Issues	PAH Exposure	POP Exposure	Metals Exposure
Birds												
Osprey	sporadic	mod	moderate	20-25	large (migratory)	nearshore	raptors	carnivore (fish)	yes	unknown	eggs	unknown
Bald Eagle	sporadic	low	moderate	up to 40 (most: 15-20)	moderate (resident)	nearshore	raptors	carnivore (fish, birds, & mammals)	yes	unknown	eggs	unknown
Surf Scoter	sporadic	moderate	moderate	unknown	moderate (mid-distance migrants)	nearshore rocky substrate	waterfowl	invertebrates	no	unknown	whole body	whole body
Marine Mammals												
Harbor Seal	yes	high	high	20-35	moderate (resdient)	nearshore	pinnepeds	carnivore (fish & invertebrates)	yes	unknown	muscle blubber	unknown
Orca	yes	low	low	50-90	large (migratory)	variable	cetaceans	carnivore (fish, marine mammals, and birds)	yes	unknown	muscle blubber	unknown

Table 1. Matrix of potential sentinal species for TBiOS monitoring

Species	Historical Chemistry Data Available	Plasma Vtg Available	Lipid Content	Liver/Gonad/Spleen Histopathology	Stomach Content Chemistry	Stomach Content Taxonomy	Hepatosomatic Index	Age/growth Data Available	Condition Index	Embryo Anomalies	Embryo Chemistry
Fish											
English Sole (adults)	extensive	yes	muscle <1%	extensive	yes	yes	yes	yes	yes	no	some ovary
English Sole (juveniles)	good	no	yes	yes	yes	yes	no	yes	yes	no	no
Pacific Staghorn Sculpin	OK: 1979-1983, 1990s	no	yes	yes	yes	yes	yes	yes	yes	no	ovary (Hg only)
Demersal Rockfish (e.g. quillback)	good, MESA/PSAMP	no	muscle <1%	yes	yes	yes	yes	yes	yes	some	ovary POPs
Ratfish (spotted ratfish)	poor	no	yes	no	no	no	no	no	no	no	no
Pacific Herring	no	no	2-10%	no	no	some	no	yes	yes	yes	yes ovaries
Herring Eggs	no	NA	≤2%	no	NA	NA	NA	NA	NA	yes	yes
Shiner Perch/Surfperch	poor	yes?	yes	yes	yes	yes	yes	yes	yes	no	small amount of POPs data
Spiny Dogfish	poor	no	yes	yes	yes	yes	yes	yes	yes	NA	no
Six Gill Shark	no	no	muscle 2-7%	no	maybe	maybe	no	maybe	yes	NA	yes
Coho Salmon (adults)	yes	yes	yes	yes	yes	yes	yes	yes	yes	NA	NA
Coho Salmon (juveniles)	some	yes	NA	no	yes	yes	yes	yes	yes	NA	NA
Chinook Salmon (adults)	yes	yes	yes	yes	yes	yes	yes	yes	yes	NA	NA
Chinook Salmon (juveniles)	yes	yes	NA	no	yes	yes	yes	yes	yes	NA	NA
Pacific Hake	poor	yes	yes	yes	yes	yes	yes	yes	yes	NA	NA
Invertebrates											
Dungeness Crab	low	NA	muscle <1%	no	no	no	no	no	no	NA	yes
Mytilus spp. (mussel)	high	NA	yes	yes	no	no	no	no	unknown	NA	

Table 1. Matrix of potential sentinal species for TBiOS monitoring

Species	Historical Chemistry Data Available	Plasma Vtg Available	Lipid Content	Liver/Gonad/Spleen Histopathology	Stomach Content Chemistry	Stomach Content Taxonomy	Hepatosomatic Index	Age/growth Data Available	Condition Index	Embryo Anomalies	Embryo Chemistry
Birds											
Osprey	some	no	unknown	no	no	yes	no	some	unknown	hatching success	egg POPs
Bald Eagle	some	no	some plasma lipid data	no	no	yes	no	some	unknown	hatching success	egg POPs
Surf Scoter	some	no	unknown	limited	no	yes	limited	some	limited	no	no
Marine Mammals											
Harbor Seal	some	NA	some	limited	from diet studies	yes	NA	some	unknown	unknown	pups
Orca	some	NA	some	limited	from diet studies	yes	NA	some	unknown	some data on pup and fetus survival	pups

Table 2. Chemicals of concern proposed for monitoring as part of TBiOS, their sources, and their potential effects in biota.

Chemical Type	Chemical Name	Source Identified in Puget Sound	Matrix to Measure	General Potential Effects in Biota
Metals	Arsenic	Runoff (Ecology, 2004) In-stream (King County, 2006)	Water column, sediment, muscle, kidney	Reduced survival of invertebrates. Malformation of marine invertebrates. Impaired growth in fish Gill hemorrhaging, necrosis or lesions of liver in fish. Death of birds (acute poisoning). Impaired growth in mammals Immune impairment in mammals Skin lesions in human Cancer in humans Reproductive effects in humans
	Cadmium	In stream (Ecology, 1994) Runoff (Davis et al. 2000)	Water column, sediment, muscle, kidney	Reduced survival of invertebrates Reproduction effects in invertebrates Reduced survival of fish Reproductive effects in fish Physiological alterations in fish Impaired growth in birds Reproductive effects in birds Teratogenic effects in mammals Decreased bone density in humans Kidney or liver dysfunction in humans
	Lead	In-stream (Ecology, 1994) Runoff (Maestre and Pitt, 2005)	Water column, sediment, muscle, kidney	Reproductive effects in invertebrates Reduced survival in fish Neurological effects in fish Developmental effects in fish Death in birds (acute poisoning) Reproductive effects in birds Cancer in humans Neurological effects in humans
	Zinc	In-stream (Ecology, 1994) Runoff (Ecology, 2006a)	Water column, sediment, muscle, kidney	Mortality in fish Skin irritations in humans (large amounts of lead)
	Mercury	In-stream (Ecology, 2004a) Runoff (Ecology, 2006a)	Water column, sediment, muscle, kidney	Altered behavior in invertebrates Brain lesions in fish Physiological alterations in fish Impaired growth in birds Developmental effects in birds Impaired growth in mammals Reproductive effects in mammals Neurological effects in mammals Neurological effects in humans

Table 2. Chemicals of concern proposed for monitoring as part of TBiOS, their sources, and their potential effects in biota.

Chemical Type	Chemical Name	Source Identified in Puget Sound	Matrix to Measure	General Potential Effects in Biota
Antifouling agent	Tributyl tin		Water column, sediment, muscle, kidney	Malformations in marine invertebrates Altered behavior in invertebrates Reproductive effects in fish Developmental abnormalities in fish Immune impairments in mammals Bone structure effects in mammals Neurological effects in humans
Persistent organic compounds	Polychlorinated biphenyls (PCBs)	In-stream (Naim, 2007) Runoff (Ecology, 2004c)	Muscle Whole body Eggs/ovaries Stomach contents	Reproduction effects in invertebrates Reproductive effects in fish Physiological alterations in fish Teratogenic effects in fish Physical deformities in birds Immune impairment in mammals Reproductive effects in mammals Cancer in humans
	Polybrominated diphenyl ethers (PBDEs)	In-stream (Ecology, 2006b)	Muscle Whole body Eggs/ovaries Stomach contents	Impaired growth in mammals Teratogenic developmental effects in mammals Liver and kidney damage effects in mammals Altered behavior in mammals Bone structure effects in mammals
	Dioxins and furans		Muscle Whole body Eggs/ovaries Stomach contents	Gill hemorrhaging or other damage in fish Tumors in fish Physiological alterations in fish Physical deformities in birds Reproductive effects in birds Impaired growth in mammals Immune impairment in mammals Cancer in humans Neurological effects in humans Cardiovascular and respiratory effects in humans.
Oil or petroleum-associated hydrocarbons	Polycyclic aromatic hydrocarbons (PAHs) (alkylated, low, and high molecular weight)	Runoff (Cullinan et al. 2005). Oil spills	Bile Stomach contents Invertebrate tissues	Reproduction effects on invertebrates Necrosis of liver lesions in fish Reproductive effects in fish Immune impairment in fish Altered behavior in fish Liver abnormalities in birds Cancer in mammals Cancer in humans

Table 2. Chemicals of concern proposed for monitoring as part of TBiOS, their sources, and their potential effects in biota.

Chemical Type	Chemical Name	Source Identified in Puget Sound	Matrix to Measure	General Potential Effects in Biota
Pesticides	Organochlorine (DDT)		Muscle Whole body Eggs/ovaries Stomach contents	Altered behavior in invertebrates Physiological alterations in fish Altered behavior in fish Reproductive effects in birds Reproductive effects in mammals Cancer in mammals Neurological effects in mammals Immune impairment in humans
	Organophosphorus and carbamate		Water column; exposure in animals may be detected by measuring	Altered behavior in invertebrates Reduced survival in fish Neurological effects in fish Physiological effects in birds Neurological effects in mammals Physiological effects in mammals Neurological effects in humans
	Pyrethroids		Muscle Whole body Eggs/ovaries Stomach contents	Paralysis of invertebrates Neurological effects in fish Neurological effects in humans Gastrointestinal effects in humans
Plasticizers	Phthalate esters		Muscle Whole body Eggs/ovaries Stomach contents	Impaired growth in fish
Emerging chemicals	Pharmaceuticals and personal care products [e.g. triclosan (antimicrobial disinfectant), caffeine (stimulant), N-N-diethyltoluamide (insect repellent)] Hormone-disrupting chemicals [e.g. bisphenol A, nonylphenol, 17b-estradiol and ethynylestradiol] Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA) Nanomaterials (nanoparticles exposure) (Oberdörster et al. 2004)	King County (200x) USGS	Plasma Bile Varies by chemical; sometime not determined	Reproductive effects in fish Physiological alterations in fish. Reproductive effects in mammals Reproductive effects in humans Neurological effects in humans Respiratory effects in humans

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Table 3. Summary of recommended available indicators, possible future indicators, and identified gaps for water quality.

Major Water Quality Goals/Outcomes	Indicator Category	Recommended Available Indicators (a)		Possible Future Indicators			Identified Gaps		
		Marine/Nearshore Habitats	Freshwater Habitats	Marine/Nearshore Habitats	Freshwater Habitats	Uplands	Marine/Nearshore Habitats	Freshwater Habitats	Uplands
Food web and ecosystem not impaired by toxics	Toxics in Biota	*Toxics in clams*		Toxics in crab and shrimp			Toxics in plankton		
		Toxics in mussels		Bioaccumulation monitoring			Toxics in geoducks		
		Toxics in marine benthic fish	*Toxics in freshwater fish - multiple sources*		Toxics in freshwater fish - air deposition				
		Toxics in marine pelagic fish							
		Fish Tissue Contaminants Index							
		Contaminants in whole fish							
		Toxics in juvenile salmon							
		Toxics in osprey eggs		Pesticide poisonings in raptors					
				Toxics in heron eggs					
				PCBs in cormorant eggs					
		Toxics In harbor seals							
	Exposure Effects	**Liver disease in English sole**		Vtg Induction in male fish			Contaminant effects on species population community and community structure		
		Sediment Quality Triad Index		Star protein/ DNA damage			Effect of emergent contaminants on ecological communities		Stormwater pollutant effects
								Toxics-related decline in amphibians	
		Marine benthic infaunal community structure							

(a) = Recommended available indicators are classified as "good" or "potential" indicators.
Good available indicators (usable in current format) are noted in **bold text**.
Potential available indicators (requiring further evaluation, a modification or expansion) are noted in *italics* .
Possible future indicators included those with little or no data to evaluate their suitability.
Indicator record numbers (shown in brackets) are taken from the Indicators Evaluation Spreadsheet.
Shaded cell contain indicators also cross-referenced in the species and food-web indicator tables (Modified from O'Neill et al. 2008).