Columbia River Toxics Reduction Working Group: Strategy For Measuring, Documenting And Reducing Chemicals Of Emerging Concern
INTRODUCTION
The Columbia River Basin (Basin) is one of the world’s great river basins. The Basin drains about 259,000 square miles across seven U.S. states and British Columbia, Canada. Of that total, about 219,400 square miles, or 85 percent of the Pacific Northwest region, are in the United States; the remaining 39,500 square miles are in Canada. The Basin’s rivers and streams carry the fourth largest volume of runoff in North America. The Columbia River begins at Columbia Lake in the Canadian Rockies and travels 1,243 miles over 14 dams to reach the Pacific Ocean a hundred miles downstream from Portland, Oregon (EPA, 2009). The Basin has been, and will continue to provide an important North American backdrop for urban settlement and development; agriculture; transportation; and recreation. The Basin also serves as a special ecosystem to many important plants and animals. Unfortunately this great watershed is also contaminated with a variety of toxic contaminants as a result of human activities. These toxic contaminants are present in air, water, and soils throughout the basin. They threaten the health of people, fish, and wildlife inhabiting the Basin.

In 2005, EPA joined with federal, state, tribal, and local governments, industry, and nonprofit partners to form the Columbia River Toxics Reduction Working Group (Working Group). The goal of the Working Group is to reduce toxics in the Basin and prevent further contamination. Since its inception the Working Group has had several accomplishments including but not limited to: 1) developing the 2009 “Columbia River Basin: State of the River Report for Toxics” (EPA 2009); 2) developing the 2010 “Columbia River Basin Toxics Reduction Action Plan” (EPA 2010); 3) organizing five workshops across the Basin on key issues such as pesticide stewardship, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs); and 4) convening two meetings of federal, tribal, state, local and non-governmental organization executives (Executives) in 2011 and 2012 to discuss how to better collaborate and further reduce toxic contamination in the Basin.

The 2010 Toxics Reduction Action Plan identified the development of a Basin-wide research plan as one of five initiatives. At the time it was recognized that while limited research on the effects of contaminants in the Basin ecosystem was being conducted by different agencies, there was no coordinated effort to identify the research priorities or gaps in our knowledge of the Basin. Since 2010, there has been little effort toward developing a research plan due to limited resources. In November 2012, the Executives met to discuss how to move forward with toxic contaminant reduction efforts. They identified six initiatives that would help to further reduce toxics including one initiative to develop and implement a research plan for Chemicals of Emerging Concern (CECs). The United States Geological Survey (USGS) agreed to coordinate the development of a research plan for CECs. The following document provides an outline of a research and monitoring strategy focusing on CECs—a portion of the larger toxic contamination issue in the Basin.
Problem Statement
CECs can include, but are not limited to, nanoparticles, pharmaceuticals, personal care products, estrogen-like compounds, flame retardants, detergents, and industrial chemicals (including those in products and packaging) (Jones and Graves 2010), and even legacy contaminants whose health effects are still emerging. A variety of CECs have been detected in the Columbia River Basin. Some examples are:

- PAHs, PCBs, and PBDEs were found throughout the lower River and in river water, sediment, and juvenile Chinook salmon. These contaminants are moving from river water and sediment into salmon prey and then into salmon tissue (LCREP 2007).
- In surficial bed sediments sampled from the lower Columbia River main stem and several tributaries, 49 different CECs were detected, with endocrine-disrupting compounds (contaminants that block or mimic hormones in the body and cause harm to fish and wildlife) detected at 22 of 23 sites sampled (Nilsen and others, 2013).
- A myriad of pharmaceuticals and personal care products were detected in the effluent from numerous WWTPs discharging to the Columbia River (Morace 2012).

Several efforts have been completed or are underway to better characterize the occurrence and impacts of CECs in the Basin. USGS and National Oceanic and Atmospheric Administration (NOAA) have completed several sampling efforts over the past five years including evaluating the impacts of estrogen-like compounds on juvenile salmon, collecting samples from several WWTPs and stormwater runoff, assessing distributions of CECs in bed sediments, and conducting research on the potential impacts of CECs on food webs and the ecosystem. In addition, the Washington State Department of Ecology and Oregon Department of Environmental Quality have conducted sampling in parts of the Basin to determine the presence of some CECs. At this time we do not know the impacts of this myriad of chemicals on the aquatic and terrestrial ecosystems in the Basin and a research and monitoring plan is required to ensure the protection of these natural resources and the people who use them.

Research Plan Strategy
The goal of this document is to provide a strategy for developing a research and monitoring plan for detecting and characterizing the biological impacts of CECs on aquatic and terrestrial wildlife in the U.S. portion of the Basin. The strategy was limited to CECs to focus the discussion, to highlight the importance of developing a better understanding of this specific class of contaminants, and to help in the prevention and reduction of toxic contaminants entering the Basin. The Working Group took this focused approach because CECs are an issue of special concern and, for the most part, little information is available on fate, transport, and impacts in the Basin.

The strategy contains the following elements:
1. Conceptual models of potential of exposure and effects in the Basin
   a. A generalized model of the Basin
b. Conceptual model of sources, fate and pathways of CECs between the biotic and abiotic compartments of an ecosystem

c. Conceptual models illustrating the flow of energy and, thus, CECs in estuarine and stream food webs

2. A list of CECs known to occur in the Basin and an example of a CEC prioritization plan
3. A description of known biological effects of CECs
4. A description of known biological indicators of exposure to CECs at various points in aquatic and terrestrial food webs.
5. A summary including how research could be used to direct specific actions or policies.

It is also important to note that the Basin CEC Work Group is working interactively with other similar groups on the West Coast (e.g., San Francisco & Puget Sound). There is a strong desire among these groups to coordinate efforts. A joint Puget Sound/Columbia River “CEC State of the Pacific Northwest” report is planned since the CEC problems and strategies are similar. This future report is intended to describe the occurrence of CECs and strategies to identify biological effects with goal to reduce CECs in both Puget Sound and the Columbia River Basin.

CONCEPTUAL MODELS

Fish, wildlife, and people are exposed to many contaminants polluting the water and sediment of the Basin. Conceptual models can be used to categorize the origins of these contaminants (sources and pathways) and the effects they may have on the ecosystem. Potential sources for these contaminants can be grouped into point sources from current and past industrial discharges to the air, land, and water and from more widespread and diffuse sources (nonpoint sources), such as runoff from farms and roads and atmospheric deposition (Figure 1). Some contaminants, such as mercury, also come from natural sources. Even when released in small amounts, some of these contaminants can build up over time to toxic levels in plants and animals (EPA 2009). While many programs focus on monitoring sentinel or charismatic species within the food web the importance of lower trophic levels should not be overlooked. The base of the food web often lacks contaminant information and in many cases may show biological impacts before they are measurable in higher trophic levels. Although the focus of this document is CECs, the conceptual models presented here are appropriate for all contaminants present in the Basin.
The Basin is a complex landscape containing multiple interacting ecosystems. In order to design an effective research and monitoring program for CECs it is important to consider questions such as:

- What are the potential sources of contaminants?
- How do they reach and move within both the abiotic (fate and transport) and biotic (food web) portions of the ecosystem?
- What are the biological impacts of the contaminants (effects)?

A generalized conceptual model for sources, fate and transport of toxic chemicals is shown in Figure 2. It is important to understand that each compartment of the system (ocean, estuary, freshwater, air, subsurface and terrestrial) will have unique components and dynamics that will need to be considered when designing a monitoring program; however, it is equally important to remember that the compartments interact at a larger scale as well. Finally, it is important to understand interactions within the food web to evaluate which species to monitor and where biological impacts might be occurring. Generalized conceptual models for food webs in an estuary (Figure 3) and a freshwater stream (Figure 4) illustrate the flow of energy (i.e., food). It
is through this flow of energy that some contaminants bioaccumulate and in some cases move to higher trophic levels to biomagnify.

Figure 2: Sources, fate and transport of contaminants between biotic and abiotic compartments (Dietrich et al. 2005, Leary et al. 2005). Arrows point to the flow of contaminants between compartments.
Figure 3: Estuarine food web (Dietrich et al. 2005, Leary et al. 2005) in which arrows point to the flow of energy (i.e., food) in the system.

Figure 4: Stream food web (Dietrich et al. 2005 and Leary et al. 2005) in which arrows point to the flow of energy (i.e., food) in the system.

BACKGROUND

Toxic contaminants are present throughout the Columbia River Basin; some are CECs that may or may not be problematic. We know they are present in the ecosystem, but we are less certain about what levels have negative effects. A contaminant may be “emerging” based on one or more of the following (1) the compound’s recent identification in the environment, (2) challenges in trying to regulate its unknown risks, (3) concern over its presence and potential effects, or (4) as a matter of scientific interest in a compound about which little or nothing is known (Arp 2012). A particular CEC may pose a real or perceived threat to human health or the environment, but there are no currently published health standards for most CECs, because the science has not advanced sufficiently to provide a basis for assessing toxicity.

CECs in the Basin.

- Brominated flame retardants (PBDEs) — PBDE body burdens in mountain whitefish, a popular sport fish in the Upper Columbia River, were doubling every 1.6 years from 1992 to 2000—faster than anywhere else reported worldwide (Rayne et al. 2003). Since this finding, PBDEs have been monitored in water, sediment, and tissues throughout the lower Basin (Johnson et al. 2006, LCREP 2007, Nilsen et al. 2014, Alvarez et al. 2014), where they are ubiquitous. Several PBDE congeners biomagnified through several levels
of the Columbia River food web (Nilsen et al. 2014), and concentrations in osprey eggs increased progressively from rural Umatilla to downstream of Portland (Henny et al. 2011). In a 2011 study, persistent, bioaccumulative, toxic chemicals, including flame retardants, were found in virtually all lower Columbia River resident fish (carp and largescale suckers) examined (Johnson and Friese 2012).

- **Estrogens**—The presence of estrogen-like compounds in the waters of the Basin is evidenced by vitellogenin induction in juvenile salmonids (LCREP 2007). Vitellogenin, an egg yolk protein normally produced in adult female fish, is an indicator of exogenous estrogen exposure when found in juvenile or male fish. Estrogen-like compounds in effluent from Portland’s WWTP (Morace 2012) are concentrated enough that the resulting estrogenicity in the Columbia River could be the equivalent of 1 ng/L estrogen—a concentration that could cause endocrine disruption in some aquatic species.

- **Pharmaceuticals and Personal Care Products**—Pharmaceuticals are expected to be in WWTP effluent because of the volume of medications used in the United States and the amount that passes through the body. As expected, a number of pharmaceuticals were found in WWTP effluent sampled throughout the Basin (Morace 2012, Rounds et al. 2009, Hope et al. 2012). These effluents are diluted when they reach their receiving waters, thus, fewer pharmaceuticals are detected in the main-stem Willamette and Columbia rivers (LCREP 2007, Alvarez et al., 2014). In contrast, many more personal-care-product ingredients were detected in surficial-bed-sediment samples collected throughout the Lower Columbia River Basin, where endocrine-disrupting compounds were detected at 22 of 23 sites (Nilsen et al. 2007; Nilsen et al. 2014). Similarly, several personal-care-product chemicals were found in WWTP effluent (Morace 2012), which raises the question of which compounds may be partitioning into the biosolids during the treatment process and at what levels.

- **Perfluorinated compounds (PFC)** — PFCs have been produced for over 50 years for use in a wide variety of industrial and consumer applications. Recently, EPA labeled a PFC and its salts “likely to be carcinogenic” (EPA, 2006). Washington State Department of Ecology found PFCs (38 to 910 ng/g) in osprey eggs collected from the Lower Columbia River (Furl and Meredith, 2010).

**Example of CEC Prioritization.**

The State of California established a science advisory panel to make recommendations for monitoring CECs in that state’s aquatic ecosystems (Anderson et al. 2012). That panel recommended that the following be used to prioritize CECs in California (Anderson et al. 2012, pages iii – iv):

1. Develop monitoring trigger levels (MTLs) for CECs that pose the greatest potential risk to aquatic systems based on published effects concentrations.
2. Compile measured or predicted environmental concentrations (MECs or PECs) for CECs for which MTLs could be estimated.
3. Identify those CECs that have the greatest potential to pose a risk by comparing MECs (or PECs) to MTLs. CECs with a monitoring trigger quotient (MTQ = MEC(or PEC)/MTL) greater than “1” were identified for monitoring. (Note than an MTQ of greater than 1.0 does not indicate a risk is present, only that sufficient potential for a risk exists that the chemical should be considered for inclusion in a monitoring program.)

4. Apply the risk-based screening framework (steps 1-3) to each of three representative scenarios that capture the key types of exposure (sources and fate) to CECs in the State’s inland, coastal and marine receiving water systems.

**Pathways and Sources.**

- **Pathways**—As illustrated in the conceptual models (Figures 1 – 4), some CECs can be linked to specific pathways (e.g., wastewater treatment plants). These pathways act as integrators of human activities and offer an area where changes could be made to lessen their effects on the environment.

- **Sources**— The term source is defined here as the object or activity from which a CEC is initially released to environmental media or released in a form which can be mobilized and transported in an environmental pathway. This definition has been adopted for the present document to distinguish the initial release of a CEC from a secondary release, such as mobilization of a chemical from a toxic cleanup site and to distinguish a source from its presence in transport/delivery pathways such as stormwater, atmospheric transport, and wastewater treatment plants. Some CECs come from unknown or diverse sources. Ubiquitous CECs, like phthalates, PBDEs and nanoparticles, present their own unique challenges when exploring reduction efforts.

**Biological Effects.**

- **Reproductive success**—Ospreys in the Basin have reduced reproductive success. In the past, this population decrease was linked to the presence of DDT and other organochlorine compounds (Henny et al. 2008). While the concentrations of these legacy compounds have been decreasing, concentrations of PBDEs and other CECs have been increasing in osprey eggs and reproductive success is once again being adversely affected (Henny et al. 2009).

- **Increased stress and bioaccumulation**—Some CECs bioaccumulate in biota, and many are suspected or known endocrine disruptors, meaning they mimic hormones and can cause problems with the endocrine system, which affects reproduction, growth, and other physiological processes. Juvenile salmonids collected near Longview, WA had low lipid content, indicative of the physiological stress of malnutrition, which may affect their ability to survive their journey to the ocean (LCREP 2007). In a food web study focused on the lower Columbia River Basin, concentrations of PBDEs, PCBs, and legacy pesticides increased in water (Alvarez et al. 2014), sediments (Counihan et al. 2014), fish tissues (Nilsen et al. 2014), and osprey eggs (Henny et al. 2011) in a downstream direction from Skamania to the more urbanized Columbia City and Longview.
Bioindicator results indicated that fish at the downstream sites experienced greater stress relative to the upstream site primarily based on kidney and liver histopathology and levels of parasitic infections; however, the stress was not linked directly to liver contaminant burden (Torres et al., 2014).

- **Reproductive and genetic impacts**—In the food web study mentioned above, reproductive variables, including spermatozoan morphologies, mitochondrial membrane potential, viability, apoptosis, ATP content, DNA fragmentation, motility and percent mature sperm, and vitellogenin (VTG) reflected negative impacts at the downstream site(s) relative to the upstream site; several variables, including total and progressive motility, live cell apoptosis, abnormal sperm morphology, ATP content, and VTG were significantly correlated with various contaminants (Jenkins et al. 2014). A gene expression microarray developed specifically for largescale suckers identified 69 genes with expression patterns that correlated with hepatic tissue levels of contaminants (Christiansen et al. 2014). Although not mechanistically tied to the exposure gradient of the three sites, these genes were involved in a range of biological processes previously shown to respond to contaminant exposure, including lipid metabolism, apoptosis, cellular transport, oxidative stress, and cellular chaperone function. Taken together, the contaminant and bioindicator results support the hypothesis that contaminants in the environment both correlate to bioaccumulation and cause genetic and reproductive impacts within the food web.

- **Long-term exposure and mixtures**—Pharmaceuticals, by intent, are biologically active, therefore, although their exact effects on wildlife are not yet fully documented, their presence in the environment would be expected to have ecological effects (Williams 2005). Pharmaceuticals and other CECs delivered through WWTP effluent can be considered to have “pseudo-persistence” because of the continual input of these compounds (Smtal 2008). The effects of continuous low-level exposure to these CECs, particularly during sensitive life stages, as well as effects of long-term exposure to these complex mixtures are further unknowns (Daughton and Ternes 1999; Han et al. 2010).

**BIOLOGICAL INDICATORS OF CEC EXPOSURE**

Biological indicators (bio-indicators) can be used to detect the presence of CECs and to assess their biological impacts. Ideally these bio-indicators will be applicable to specific classes of compounds and can be measured in individual organisms but have validated implications at the level of the population, community or ecosystem. To encompass the wide range of chemicals and associated types of biological injury that may occur in natural populations, a suite of bio-indicators is needed to identify chemical exposure, as well as the effects of chronic and acute exposure. A good bio-indicator should be applicable in laboratory research, field studies and monitoring programs. Bio-indicators are an essential component of any CEC monitoring program, because they can tell us not only that these contaminants are present in the environment but also that they are actually being absorbed by and affecting fish and wildlife. Biological monitoring helps us to understand the significance of the concentrations of contaminants we measure in the environment, and often the effects on biota can identify problems that water and sediment monitoring do not detect.
Bio-indicators can be classified into various categories: (1) enzymes involved in the metabolism of toxic contaminants, and serve as indicators of exposure, (2) biochemical or physiological responses such as changes in plasma chemistry or hormone levels, or (3) true injury that could have implications on the population level, such as impairment of growth or reproduction.

Appendix 1 lists a number of bio-indicators that have been used in field assessments to monitor contaminant exposure and effects in fish and wildlife. The following is a summary of bio-indicators used in numerous studies in the Basin. Similar studies have been conducted in other parts of the country but are too numerous to include. The following studies measured a wide range of land use variables (e.g., urbanization, agriculture) and contaminants (not limited to CECs) and relate these to bio-indicators that might be of value in a CEC research and monitoring program. These bio-indicators include metrics that range from the community to the molecular level of biological organization. However, no studies specifically address changes in the macroinvertebrate community associated with CECs.

**Macroinvertebrate Community.**

- In an evaluation of benthic community structure in parts of Oregon and Washington (Bi-State Study, Tetra Tech 1996), approximately half of the communities sampled had reduced diversity compared to reference conditions, but there were no significant correlations between contaminant concentrations and taxa richness or abundance. Several additional studies in the Willamette and Columbia rivers have examined changes in macroinvertebrate community structure with urbanization or chemical contamination.
- Waite et al. (2008) described the effects of urbanization on biological characteristics of stream ecosystems in 28 watersheds along a gradient of urbanization in the Willamette River basin and surrounding area, sampled from 2003 through 2005. They found that sensitive macroinvertebrate taxa richness and abundance and intolerant taxa abundance declined with increasing urbanization, total pesticide concentrations in the water, and aryl hydrocarbon toxic equivalents (TEQs) in water. Aryl hydrocarbon TEQs were estimated from a P450 reporter gene system for aryl hydrocarbon receptor agonists (e.g., PAHs, PCBs, dioxins and furans) assayed on extracts from semi-permeable membrane devices.
- Bortleson et al. (1994) examined the relationship between benthic invertebrate community structure and distribution of trace elements and wood-pulp-related compounds in the sediments of Lake Roosevelt and the upstream reaches of the Columbia River. Benthic invertebrate communities in the Columbia River showed effects from trace elements in bed sediments.
- Flinders et al. (2009) examined changes in macroinvertebrate communities exposed to pulp and paper mill effluent in the Willamette River using a range of metrics as bio-indicators including % dominant taxa, density, richness, Hilsenhoff Biotic Index [HBI], Simpson's Index, and ash-free dry mass. While some significant inter-site differences were observed, they were not related to pulp mill effluent levels.
- Between 1994 and 2010, Hope et al. (2012) used data on trace metals and organic chemicals in surface waters of the Willamette River and vertebrate- and invertebrate-
assemblage indices to correlate the presence of chemical stressors with biological impacts. The results suggested that land use practices (i.e., increased agricultural or urban land use) and chemical mixtures (primarily pesticides) may have impacted the basin’s aquatic ecosystems.

- A number of other studies have collected information on macroinvertebrate assemblages in the Columbia and Willamette rivers (e.g., Jones et al. 1981, 1990; McCabe et al. 1997, 1998, Furota and Emmett 1993; Sagar et al. 2013) but do not specifically correlate these with measures of contamination or urbanization.

**Fish Health.**

*Fish community evaluations.*

- The Bi-State Study included a fish community assessment based on the Index of Biotic Integrity (IBI; Karr et al. 1986) that used 12 bio-indicators (e.g., number of fish species, presence of native vs. exotic species, percent anomalies, species tolerance) to assess integrity based on the fish community’s taxonomic and trophic composition. Although there were some difficulties in collecting sufficient fish to calculate the IBI for all river segments, lower IBI scores were observed in the segment of the Columbia River between the Cowlitz and Willamette river confluences, where exceedances of reference levels for pollutants were most frequent. Similar work was carried out in the Willamette River (Hughes and Gammon 1987; TetraTech 1993, 1994). Hughes and Gammon (1987) observed a downstream gradient in IBI in the Willamette River associated with declining water quality, as well as declines in IBI near point sources of pollution. They also observed increased disease and morphological abnormalities and decreased biomass in fish in the lower river.

- Flinders et al. (2009) examined patterns of fish community structure in relation to pulp mill discharges in the Willamette River. Fish metrics used as bio-indicators included species abundance, species richness, Simpson’s diversity, % dominant species, standing crop, % fish with deformities, fin erosion, lesions or tumors (as assessed by gross examination), % intolerant, % omnivore, and % piscivore. However, pulp mill effluent exposure appeared to have little effect on fish communities in the Willamette River.

- Waite et al. (2008) described the effects of urbanization on fish assemblages in the Willamette River and found that a single species was more likely to be dominant as urbanization increased, and a fish bio-indicator combining percentages of salmonids, reticulate sculpins, nonnatives, and natives, was highly correlated with urbanization and TEQs. Generally, the most urbanized sites had the highest abundances of nonnative fish species and lower abundances of sensitive salmonids.

**Pathological conditions in fish.**

- An autopsy-based bio-indicator was used on largescale suckers following the U.S. EPA Rapid Bioassessment Protocol V (Plafkin et al. 1989) that characterized the appearance of the exterior and interior tissues and organs (e.g., thymus, pseudobranch, gills, kidney, spleen, liver) and measured blood hematocrit, leucocrit, and plasma protein levels (Tetra Tech 1996). This assessment did not yield any clear spatial patterns in fish health.
measures related to urbanization or concentrations of specific contaminants but provides some baseline information on bio-indicators.

- The USGS conducted another large-scale study in 1997 and 1998 as part of its Biomonitoring of Environmental Status and Trends (BEST) program (Hinck et al. 2004, 2006). This project examined 560 fish representing 8 species from 16 stations in the Basin and relied on bio-indicators such as gonad histopathology, activity of the toxicant metabolizing enzyme, ethoxyresorufin-0-deethylase (EROD), liver and spleen macrophage aggregate analysis, vitellogenin (VTG) induction, somatic indices, and results of a necropsy based health assessment similar to that conducted in the Bi-State study (Tetra Tech 1996). Common carp, largemouth and smallmouth bass, and largescale sucker accounted for 80% of fish sampled in the study. Significant findings relative to effects of CECs were ovotestes in male bass from Lewiston, ID and Warrendale, OR, and VTG induction in male bass, with particularly high levels at Creston, MT, Grand Coulee, WA and Pasco, WA.

- Juvenile white sturgeon from the Portland Harbor in the Willamette River were examined visually for gross external abnormalities (as part of the Superfund Remedial Investigation; LWG 2011), and 55% had external anomalies of the body, head, eyes, opercles, or gills. This was slightly greater than the prevalence of abnormalities (25 to 46%) observed in Basin fish (carp, bass, largescale sucker, northern pikeminnow, longnose sucker, walleye, and rainbow trout) by Hinck et al. (2004).

- Tetra Tech (1996) examined skeletal deformities in juvenile fish from the Columbia River, and several other studies have addressed this problem in fish from the Willamette River (e.g., TetraTech 1994; Ellis 2000; Markle et al. 2002; Villeneuve et al. 2005). However, the most recent work (Villeneuve et al. 2005) suggests these deformities may be due to a parasitic infection rather than exposure to chemicals such as CECs.

- There have also been some surveys of pathogens in fish from the Columbia River. Arkoosh et al. (2004) monitored the prevalence of various pathogens in juvenile Chinook and coho salmon from Pacific Northwest estuaries, including the Columbia River estuary, while Van Gaest et al. (2011) screened the kidneys of emigrating Snake River spring Chinook salmon for pathogens. However, these studies did not link these bio-indicators with contaminant exposure. There are also laboratory studies suggesting CECs in the Columbia River have the potential to affect the health of fish and other aquatic organisms. For example, Arkoosh et al. (2010) demonstrated changes in disease resistance in juvenile Chinook salmon fed a diet contaminated with PBDEs at concentrations similar to those measured in stomach contents of juvenile salmon from Portland Harbor.

**Biochemical and physiological indicators.**

- Biochemical indicators of contaminant exposure and effects have been measured in several Columbia and Willamette river studies. EROD induction-related measures of cytochrome P450 (CYP1A) activity were examined in largescale suckers (Tetra Tech 1993, 1994; Collier et al. 1996) and in several other resident fish species in the USGS Columbia River BEST study (Hinck et al. 2004, 2006). However, EROD is indicative of
exposure to contaminants such as PAHs, dioxins and furans, and dioxin-like PCBs and is not specific to CECs.

- Hinck et al. (2004, 2006) measured vitellogenin (VTG) induction in resident fish from several Columbia River sites and observed VTG induction in male bass, with particularly high levels at Creston, MT, Grand Coulee, WA and Pasco, WA. VTG induction was also reported in juvenile Chinook salmon from the Lower Columbia River as part of the Estuary Partnership Water Quality and Salmon Study (Estuary Partnership 2007), with especially high levels in fish from the Portland area. The VTG induction indicates that these fish are exposed to estrogenic substances, but as yet there is no information on exposure to specific pharmaceuticals and personal care products in fish from these sites.

- Some work has been done on bio-indicators of reproductive success in white sturgeon from the Columbia River showing depressed sex steroid hormones and gonadal lesions in these fish (Foster et al. 2001a, 2001b; Feist et al 2005). These abnormalities were correlated with concentrations of organochlorine pesticides and PCBs in tissues, but CECs were not measured.

- As part of the Lower Columbia River Ecosystem Monitoring Project, considerable data on condition factor, growth rate, and lipid content were collected in juvenile Chinook salmon, as well as information on accumulation of several persistent pollutants, including PBDEs, which are widespread in the lower river (LCREP 2007; Sloan et al. 2010; Yanagida et al. 2011; Johnson et al. 2013; Sagar et al. 2013).

- Arkoosh et al. (2011) also provided information on lipid content, lipid classes, and persistent organic pollutants, including PBDEs, in outmigrant yearling Snake River spring Chinook salmon released from Idaho hatcheries.

- A number of additional studies have collected information on physiological indicators of fish health (e.g. growth rates as estimated from otoliths or RNA:DNA ratios, body lipid content and classes, plasma chemistry measures such as albumin, calcium, cholesterol, triglycerides, glucose, and lipase) in juvenile salmon and resident fish species from the Snake and Columbia river systems (Congleton and Wagner 2004, 2006, Diefenderfer et al. 2011; Hatch et al. 2011) but did not provide concurrent information on chemical contaminant exposure. Diefenderfer et al. (2011) for example, provided information on a wide variety of physiological bio-indicators in juvenile salmon from main channel and off channel habitats and observed some differences between the two groups. This pilot study did not include the range of sites needed to link these differences to urbanization, industrialization, or possible exposure to contaminants.

- Bioindicator results from Torres et al. (2014) indicated that fish near Longview (in an urban area) and Columbia City (downstream of the urban site) experienced greater stress relative to an upstream site near Skamania Landing primarily based on kidney and liver histopathology and levels of parasitic infections; however, the greater stress was not statistically linked to hepatic contaminant concentrations.

- Reproductive parameters, including spermatozoan morphologies, mitochondrial membrane potential, viability, apoptosis, ATP content, DNA fragmentation, motility and percent mature sperm, and vitellogenin (VTG) reflected negative impacts at the same sites near Columbia City and Longview relative to the upstream Skamania site. Several
parameters, including total and progressive motility, live cell apoptosis, abnormal sperm morphology, ATP content, and VTG were significantly correlated with various legacy contaminants and CECs (Jenkins et al. 2014).

**Molecular indicators**

- Scientists have been using gene expression microarrays developed for largescale suckers to relate contaminant levels to biological effects at the molecular level. The expression patterns of 69 genes correlated with liver tissue levels of contaminants (Christiansen et al., 2014). The expression of these genes has not yet been correlated to specific effects, but these genes are involved in a range of biological processes previously shown to respond to contaminant exposure, including lipid metabolism, apoptosis, cellular transport, oxidative stress, and cellular chaperone function. In future studies, these genes may form the basis for monitoring exposure of fish and other wildlife to contaminant mixtures found in the Basin.

- Connon et al. (2012) applied molecular profiling using quantitative polymerase chain reaction (qPCR) technology to emigrant juvenile steelhead trout from the Snake River to assess the sublethal effects of environmental stressors. The study monitored the expression of genes associated with immune system responses and pathogen-defense (NRAMP, Mx, CXC); general stress (HSP70); metal-binding (metallothionein-A); and xenobiotic metabolism (Cyp1a1) in the gill and head kidney. Gene profiles correlated well with the presence of pathogens and a health assessment based on visual examination.

**Wildlife Health Assessments.**

- As part of the Bi-State study, health assessments were conducted for otter, mink, and bald eagles (TetraTech 1996). Otter showed histopathological effects in liver and testes and reduced baculum (penis bone) size in animals with high concentrations of PCBs and persistent organic pollutants. The most severe effects were found in animals from Columbia River mile 119.5 near an aluminum refining facility in Oregon and a pulp and paper mill in Washington. In the same study, bald eagle eggs had concentrations of DDE, PCBs, and 2,3,7,8-TCDD at levels that could reduce breeding success; there was also evidence of eggshell thinning. However, previous data showed a general trend of decreasing contaminants and improved breeding success in Columbia River eagles. Several additional studies have linked impaired health in osprey, cormorants, bald eagles, and mink with exposure to contaminants (Harding et al. 1999; Elliott et al. 2001; Buck et al. 2005; Henny et al. 2008, 2009, 2011).

- Most of these studies focused on PCBs and organochlorine pesticides, with no data on CECs. Exceptions are recent osprey studies documenting uptake of PBDEs and PFCs in eggs of these birds (Henny et al. 2009, 2011, Furl and Meredith, 2010). Bio-indicators measured in these studies include gonadosomatic index and other condition indices, histopathology, body fat levels, egg production and hatching, eggshell thickness, growth rates, as well as EROD activity and vitamin A levels.
• The effects of PBDEs on thyroid hormones have been studied in bald eagles from British Columbia and California (Cesh et al. 2010) but not in the Columbia River.
• Bryce et al. (2002) developed a bird integrity index (BII) using bird assemblage information to assess human impacts on 13 stream reaches in the Willamette River Basin. This bio-indicator includes 13 metrics representing factors such as bird taxonomic richness, tolerance or intolerance to human disturbance, dietary preferences, foraging techniques, and nesting strategies. The BII was sensitive to measures of site disturbance representative of urban impacts, such as land use/land cover, road density, riparian cover, and stream channel and substrate conditions, and correlated fairly well with a fish index of biological integrity (IBI) and two benthic macroinvertebrate metrics. However, chemical pollutants, including CECs were not specifically addressed.

CONCLUSIONS

A better understanding of the sources, pathways and effects of exposure to CECs in the Columbia River Basin is needed to develop reduction efforts and restore ecosystem health. Research and monitoring programs are essential to improving our understanding. The biological effects of many of these contaminants are not well understood, so this is an area where research is needed to develop guidelines for what levels of specific CECs actually constitute threats.

Of the studies and bio-indicators presented in this document, some are especially relevant to advancing our understanding of CECs in the Basin, including studies of PBDE concentrations and effects on thyroid function and disease resistance in fish and birds; studies monitoring vitellogenin induction in fish as an indicator of exposure to environmental estrogens; data on reproductive parameters such as hormone levels, sperm morphology, and gonad histopathology (including ovotestes) in fish; data on physiological indicators such as growth and lipid content that could be affected by CECs; and microarray and related gene expression studies that may identify changes in gene expression associated with exposure to CECs. Our challenge will be to identify the most appropriate set of indicators to use in a research and monitoring program to target this diverse set of contaminants.

In conclusion, we are confronting a new challenge in protecting human health and the environment. This challenge is complex and requires new information to effectively manage the evolving suite of emerging contaminants. Our first steps in addressing these needs should include:

• Determine the initial list of CECs upon which to focus,
• Decide upon criteria with which to prioritize CECs
• From the prioritized list of CECs, determine their specific sources, the amounts released and the pathways into the abiotic and biotic compartments of the ecosystem
• Establish guidelines for determining the level at which a specific CEC constitutes a threat to environmental health
• Establish a standardized research approach to assess the potential impacts of CECs
• Establish a standardized monitoring program through which to track CEC pathways and assess their biological effects.

The better understanding of CECs that this suite of actions will provide will allow society to develop reduction efforts and restoration plans to reduce threats to human and environmental health.
REFERENCES


Collier, T.K., B.F. Anulacion, and J.E Stein. 1996. Assessment of exposure to aromatic compounds in fish from the Lower Columbia River, by use of appropriate biomarkers. Prepared for the Oregon Department of Environmental Quality, Portland, OR by the NOAA Fisheries Northwest Fisheries Science Center, Environmental Conservation Division, Seattle, WA.


Flinders et al. 2009


Johnsen A and Friese M. 2012. PBTs analyzed in bottom fish from four Washington rivers and lakes: hexabromocyclododecane, tetrabromobisphenol a,chlorinated paraffins,polybrominated diphenylethers, polychlorinated naphthalenes, perfluorinated organic compounds, lead, and cadmium. Washington Department of Ecology publication No. 06-03-027, 116 p.


McCabe, GT Jr; Hinton, SA; Emmett, R L; Sandford, B P. Benthic invertebrates and sediment characteristics in main channel habitats in the lower Columbia River Northwest Science71. 1 (Feb 1997): 45-55.


Nilsen EB, Zaugg SD, Alvarez DA, Morace JL, Waite I, Counihan T, et al., 2014. Contaminants of legacy and emerging concern in largescale scucker (Catostomus macrocheilus) and the food


Torres, L.E., 2011, Laboratory and field assessments of brominated flame retardants and other environmental contaminants—Effects in Fishes: Dissertation of Biology, Texas Tech University, 176 p.


Appendix 1. Bioindicators used in toxicological monitoring studies

<table>
<thead>
<tr>
<th>Category</th>
<th>Bioindicator</th>
<th>Related contaminants</th>
<th>Used in Columbia River</th>
<th>Exposure/Response/Effect</th>
<th>Toxicant specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure indicator</td>
<td>Vitellogenin levels in juvenile and male fish</td>
<td>Estrogenic compounds</td>
<td>Yes</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Growth and condition indicator, general health</td>
<td>Plasma chemistry (e.g. albumin, calcium, cholesterol, triglycerides, glucose, lipase)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Growth and condition indicator</td>
<td>Growth (otolith)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Growth and condition indicator</td>
<td>Condition factor (CF)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Growth and condition indicator</td>
<td>Lipid content</td>
<td>Multiple</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Growth and condition indicator</td>
<td>Thyroid hormones</td>
<td>PCBs, PBDEs, possibly others</td>
<td>Yes</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Growth and condition indicator</td>
<td>IGF</td>
<td>Multiple</td>
<td>Yes</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Immunocompetence; general health</td>
<td>Health (presence of disease or infection)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Indicator of</td>
<td>Carboxyleste Pyrethoids,</td>
<td>?</td>
<td>Response</td>
<td>2?</td>
<td>2?</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism, liver cell proliferation</td>
<td>Liver somatic index (LSI)</td>
<td>PAHs, PCBs, others</td>
<td>?</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------</td>
<td>-------------------</td>
<td>---</td>
<td>--------</td>
<td>---</td>
</tr>
<tr>
<td>Indicator of mutagenicity</td>
<td>DNA damage (comet assay)</td>
<td>PAHs, others</td>
<td>?</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Indicator of mutagenicity</td>
<td>DNA damage (DNA adducts)</td>
<td>PAHs</td>
<td>?</td>
<td>Effect</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism</td>
<td>Metabolites of CECs in fish bile</td>
<td>Multiple</td>
<td>?</td>
<td>Exposure</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism</td>
<td>Ethoxyresorufin O-deethylase (EROD) activity/ cytochrome p450 levels</td>
<td>PAHs, dioxin-like PCBs, dioxins</td>
<td>Yes</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism</td>
<td>Acetylcholinesterase (AChE) activity</td>
<td>organophosphates</td>
<td>Yes</td>
<td>Response</td>
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</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism</td>
<td>Metallothionein levels</td>
<td>Metals</td>
<td>Yes</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism</td>
<td>Aryl hydrocarbon receptor (AhR) levels</td>
<td>PAHs, dioxin-like PCBs, dioxins</td>
<td>?</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism, oxidative stress</td>
<td>Glutathione S-transferase (GST) activity</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism, oxidative stress</td>
<td>Glutathione peroxidase (GPx) activity</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td>----------</td>
<td>---</td>
<td>----------</td>
<td>---</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism, oxidative stress</td>
<td>Glutathione reductase (GR) activity</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Indicator of toxicant exposure and metabolism, oxidative stress</td>
<td>Glutathione (GSH)</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>1</td>
</tr>
<tr>
<td>Multiple effect and exposure endpoints</td>
<td>Gene expression (genomic profile or specific genes)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Superoxide dismutase (SOD) activity</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Catalase (CAT) activity</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>2</td>
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<tr>
<td>Oxidative stress</td>
<td>Reactive oxygen species (ROS)</td>
<td>Multiple</td>
<td>?</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Lipid peroxidation (LPO)</td>
<td>Multiple</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Contaminant and environmental stress</td>
<td>Hsp70 levels</td>
<td>Multiple</td>
<td>Yes</td>
<td>Response</td>
<td>2</td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td>Gonadosomatic index (GSI)</td>
<td>Endocrine disruptors</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
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<tr>
<td>Reproductive</td>
<td>Gonad</td>
<td>Endocrine</td>
<td>Yes</td>
<td>Effect</td>
<td>2</td>
</tr>
<tr>
<td>Indicator</td>
<td>histopathology disruptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------</td>
<td>--------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitellogenin levels in maturing female fish</td>
<td>Endocrine disruptors Yes Response 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>StAR protein</td>
<td>Endocrine disruptors No Response 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex steroid levels (estradiol-17β, testosterone, 11-ketotestosterone)</td>
<td>Endocrine disruptors Yes Response 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH and FSH</td>
<td>Endocrine disruptors ? Response 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive Indicator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatase activity</td>
<td>Endocrine disruptors ? Response 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxicopathic disease, cancer</td>
<td>Liver histopathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primarily PAHs for neoplasms and pre-neoplasms; other contaminants as well for degenerative and proliferative lesions</td>
<td>Yes Effect 1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1- Association with toxicant exposure well documented and described.
2- Affected by multiple factors, including contaminants.
Columbia River Toxics Reduction Working Group:

Strategy For Measuring, Documenting And Reducing Chemicals Of Emerging Concern