AN ABSTRACT OF THE DISSERTATION OF

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Title: Passive Sampling Devices as Biological Surrogates for Evaluating Seasonal Bioavailability of Hydrophobic Organic Contaminants in Surface Water.

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Kim A. Anderson

The seasonal distribution of bioavailable organochlorine contaminants in surface water and the potential environmental factors influencing their bioavailability were evaluated. The study was carried at the lower Willamette River at Portland Harbor, Oregon where surface water runoff varied according to season. Bioavailable water concentrations of DDTs and PCBs were determined using a polyethylene membrane containing passive sampling device (PSD), known as semipermeable membrane device (SPMD). Our findings indicated that the influence of river seasonality on the bioavailable distributions of organochlorine contaminants was compound- and site-specific. Bioavailable ΣDDTs concentrations were strongly affected by the local historic use of DDTs and seasonal changes in river conditions. The dominance of bioavailable p,p'-DDD and large DDD/DDE ratios observed during low flow condition in summer suggest redistribution of p,p'-DDD into the water column and conditions favoring reductive dechlorination of p,p'-DDT to p,p'-DDD. In contrast, bioavailable dieldrin and ΣPCB concentrations were significantly increased during high flow condition in fall, especially during episodic rainstorm events. While a discernable seasonal pattern for PCBs was observed along the 18-mile stretch study area, the seasonal pattern of dieldrin was only apparent at the sampling site downstream of an agricultural creek with historical use of dieldrin. The increase in bioavailable PCB concentrations and daily loads coincident with high precipitation and sewer overflows in fall suggested a significant contribution of PCBs from precipitation input and urban storm water discharges to the surface water. Seasonal bioavailable concentrations of organochlorine compounds exceeded

the national and the Oregon water quality criteria revealing the significance of considering realistic seasonal and site-specific influences on bioavailable organochlorine distributions when performing risk assessments. In addition, we developed the triolein-free polyethylene lay flat tubing (LFT) as an alternative *in situ* PSD. The LFT proved reliable and had the same benefits as SPMD, but was simpler, inexpensive and lacked interference from the triolein impurities. The LFT tended to accumulate compounds with high log K_{ow} faster than SPMD. LFT sampling rates were estimated and modeled for 33 target analytes, including PAHs, PCBs, and organochlorine pesticides. The successful determination of field derived data illustrates the effectiveness and reliability of LFT for environmental monitoring.

Passive Sampling Devices as Biological Surrogates for Evaluating Seasonal Bioavailability of Hydrophobic Organic Contaminants in Surface Water

by
Doolalai Sethajintanin

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APPROVED:

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Major Professor, representing Toxicology

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Head of the Department of Environmental and Molecular Toxicology

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University Libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Doolalai Sethajintanin, Author

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CONTRIBUTION OF AUTHORS

This study was conducted with the contributions of the following co-authors. Dr. Kim A. Anderson provided guidance on all aspects of this dissertation. Eugene R. Johnson participated in the preparation of triolein-free low density polyethylene membrane in Chapter 3 and the chemical analysis work in Chapter 4.

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CHAPTER 1

INTRODUCTION

Understanding the distribution of chemicals in the environment is required to assess exposure to humans and biota. The environmental transport of hydrophobic organic contaminants such as polychlorinated biphenyls (PCBs), p,p'-DDT (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane) and polycyclic aromatic hydrocarbons (PAHs) are dependent on their distribution properties among various environmental phases. These distribution characteristics are modeled by phase-distribution equilibrium constants such as water solubility, octanol-water partition coefficient (K_{ow}), sorption coefficient (K_d or K_{oc}), vapor pressure (V_p), and air-water partition coefficient (K_H)(*1*). In aqueous environments, these contaminants exist in diverse states in surface water. They can be freely dissolved in surface water or associate with dissolved or particulate organic matter depending on their chemical and physical properties (*2*). All of these forms can be transported by water or distributed throughout surface water and their concentrations may exert adverse effects on aquatic organisms and human health through multiple sources and pathways with variable and often poorly understood factors.

The freely dissolved form of hydrophobic organic contaminants is considered environmentally relevant to their bioavailability, toxicity, mobility and degradation processes (*3-5*). It is the freely dissolved form of the contaminant which is transported across biological membranes of aquatic organisms and potentially exerts toxic effects (*2, 4, 5*). A reduction in the freely dissolved concentration of the contaminant refers directly to reduced bioavailability. However, there is no clear distinction between the processes that control the distribution of chemical contaminants in an environment and those that directly affect bioavailability (*6*). Spatial and temporal factors, including seasonal changes in physical,

chemical and biological aspects of the aquatic ecosystem, may modify contaminant bioavailability (i.e., aqueous activity and fugacity) and change the concentration available for uptake by aquatic organisms. The potential uptake of toxic contaminants is affected by changes of either the concentrations of the contaminant in the organism's immediate environment or the environmental bioavailability of the contaminant (*6*).

Bioavailability is a key to determining the nature of the exposure and the toxicity of a chemical in the environment. However, it is a problematic parameter to measure. Bioavailability, by definition, is determined by target concentrations in organisms (*5*). Any chemical estimates of bioavailability must be correlated with biological measures of bioavailability or toxicological bioavailability (*5*). Recent developments in passive sampling devices provide an alternative to understand chemical bioavailability (*7*). Instead of measuring the total concentration in an environmental medium, the passive sampling technique measures the concentration in a reference phase which can be brought into equilibrium with the medium (*7*). The availability of a chemical is measured based on the chemical potential which is logarithmically related to its fugacity and linearly related to its freely dissolved concentration in a particular medium (*7*). As correlated with biological concentrations, chemical estimates using passive sampling devices can predict toxicity and can be used as a screening tool in the first tier of ecological risk assessment.

Identifying and measuring the chemical forms that are actually the bioavailable forms is necessary to better understand the physical, chemical, and biological mechanisms that control contaminant fates and effects in the aquatic environment. Advanced understanding of bioavailable chemical distribution is required to predict accurately the fate and effects of environmental contaminants and this knowledge can have substantial benefits for exposure assessments and water-quality regulations. Despite the importance of environmental bioavailability, very few studies have actually evaluated the distribution of chemical bioavailability and the influencing factors in the field under different and often more variable geochemical or physiological conditions than in the laboratory.

As a part of an assessment of the bioavailability of a chemical contaminant, the ultimate goals of this dissertation were to understand the seasonal distribution of bioavailable

hydrophobic contaminants in surface water and to evaluate the potential environmental factors influencing their bioavailability. In addition, further development of a passive sampling device to measure bioavailable hydrophobic contaminants and field trial were conducted and its efficiency was compared to a widely used passive sampling device. The dissertation chapters are described below.

This chapter (Chapter 1) contains a general introduction providing background information needed for the study. It contains the basic concept of bioavailability, passive sampling technique, and general background of the target analytes and study area. The dissertation hypotheses and objectives are present at the end of the chapter.

Chapter 2 presents the field study of distribution and seasonal variation of bioavailable organochlorines in surface water using passive sampling techniques. The study was carried out at the lower Willamette River at Portland Harbor, Oregon where surface water runoff is determined by climatic influence and varies according to season (*8*). The study area (River Mile 1 to18) included the Portland Harbor superfund site (River Mile 3.5 to 9.5) and the McCormick Baxter superfund site (River Mile 7 East) (*9*). The potential environmental factors influencing their bioavailable concentration distributions are discussed. To screen the potential impacts associated with seasonal changes on aquatic organisms, the bioavailable concentrations are compared to the national recommended water quality criteria (*10*) and the Oregon water quality criteria (*11*).

Chapter 3 presents the further development of a passive sampling device. The field trial of triolein-free low density polyethylene membrane was studied. Its efficiency was compared to the widely used passive sampling device. The sampling rates and uptake model for a wide range of hydrophobic contaminants are proposed.

Chapter 4 is part of a collaborative study of "Environmental stresses and skeleton deformities in fish from the Willamette River, Oregon" (*12*). This chapter focuses only on the distribution of bioavailable PCBs and organochlorine pesticides along specific sections of the Willamette River (Newberg Pool, River Mile 44 to 47), where elevated frequencies of skeletal deformities in fish were detected, relative to those in the upper river (Corvallis, River

Mile 135). The complete collaborative work has been published in *Environmental Science and Technology. Volume 39, No. 10, 2005, page 3495-3506, "Environmental stresses and skeletal deformities in fish from the Willamette River, Oregon" (12)*, see Appendix E.

Chapter 5 summarizes the overall conclusion of this dissertation. The direction for future work is also provided.

Background

Bioavailability. Bioavailability of a contaminant is specific to the exposure matrix, duration of exposure, route of entry, and receptor. The concept of bioavailability consists of three major components (*13*) as described in Figure 1.1. Environmental availability represents the portion of the total material present in a compartment or compartments of the environment which actually involves a particular process or group of processes and is subject to modifying influences (*13*). However, the total amount of chemical potentially available is not necessarily involved in a given process. Bioavailability is a special case of environmental availability when organisms are involved in the process as a target. Bioavailability may be described as the portion of the total quantity or concentration of a chemical in the environment or a portion of it that is potentially available for biological action, such as uptake by an aquatic organism (*14*). Toxicological bioavailability is the fraction of the dose absorbed by the organism which reaches the target sites in the organism or effective intercellular concentration in the organism (*13*).

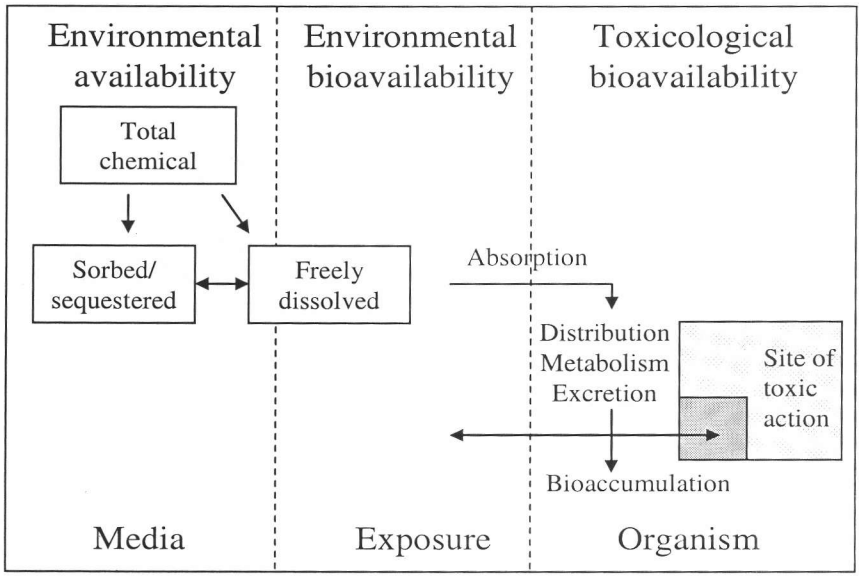


Figure 1.1 Schematic representation of bioavailability as modified from Dickson et al. 1994 (*13*) and Wells and Lanno 2001(*15*).

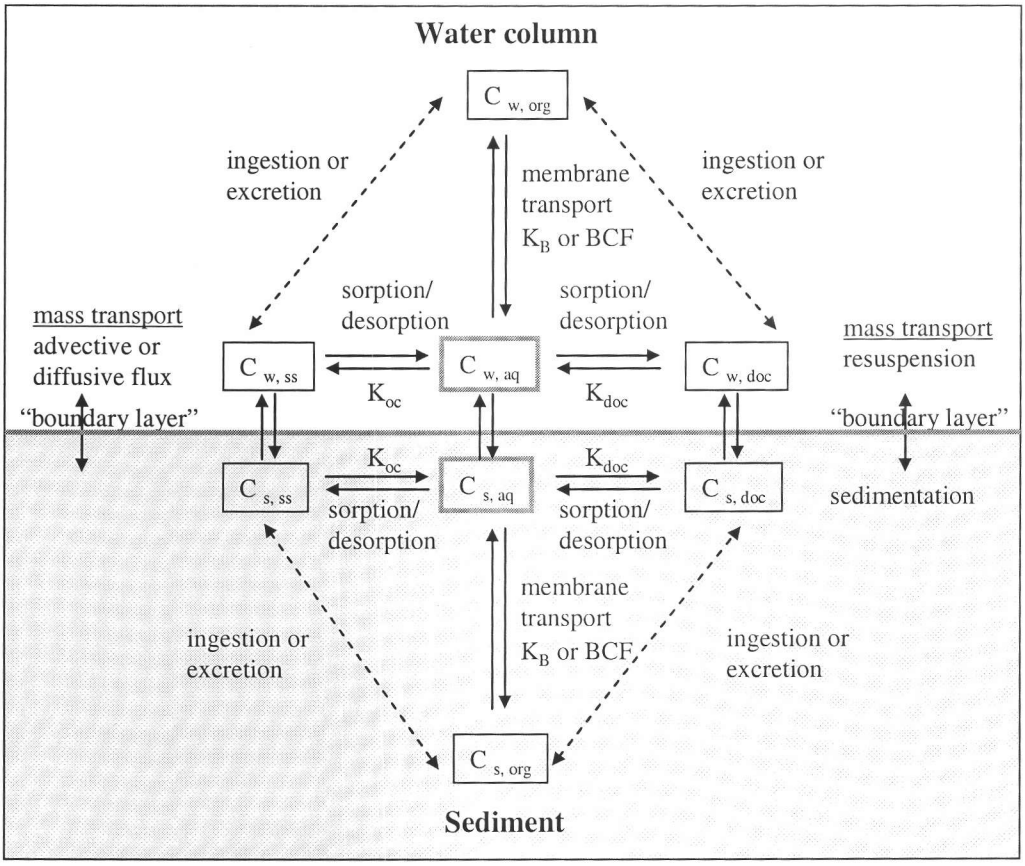


Figure 1.2 A simple four-phase model describing the bioavailability and the partitioning approach to the distribution of organic contaminant in water-sediment systems. The freely dissolved fractions of the contaminants ($C_{w,aq}$ and $C_{s,\,aq}$) are the most bioavailable. The contribution from bound fractions ($C_{w,ss}$, $C_{w,doc}$, $C_{s,ss}$ and $C_{s,\,doc}$) may become important for certain organism via ingestion as part of the food supply. The first subscript indicates the environmental compartment; w = water, s = sediment. The second subscript refers to the phase within that compartment; aq = aqueous (freely dissolved), org = organism, doc = dissolved organic carbon, ss = suspended solids. (modified from Suffet et al. 1994 (*2*))

In aquatic systems, if the uptake through ingestion of food is excluded, the freely dissolved fractions of organic contaminants are the most bioavailable (*5*). Organic chemicals that are freely dissolved in the water, and not bound to or associated with organic matter, are subject to diffusion transport across aquatic organism's membrane from the external aqueous (*2*). Figure 1.2 illustrates a simple four-phase model describing the bioavailability and the partitioning approach to the distribution of organic contaminant in water-sediment systems.

The aqueous concentration at equilibrium is related to the distribution coefficient (K) of the various phases, assuming the interactions of the contaminant concentrations are at equilibrium in the water column and sediment. Partitioning to sediments, and dissolved or particulate organic matter decreases freely dissolved concentration of the contaminant and thus the bioavailability of contaminants to biota (*2, 5*). Many factors can influence the environmental availability including the environmental bioavailability by altering the environmental distribution of the chemicals and this would consequently alter bioconcentration and bioaccumulation (*2, 6, 14*). Physical factors include temperature, advection, and sedimentation and resuspension. Physicochemical factors include dissolution, desorption, diffusion, and degradation. Biological factors include diagenesis, organic matter loading and bioturbation. However, there is no clear distinction between the processes that control the distribution of chemical contaminants in an environment and those that directly affect bioavailability (*6*).

Significance of spatial and seasonal aspects on chemical distribution. A consideration of the spatial and seasonal aspects is essential to chemical exposure in ecological risk assessment (*16*). These variables affect the concentrations available for uptake from surrounding media by organisms. Several studies have indicated bioaccumulation of PCBs and DDTs in fish and lower trophic level biota seasonally changed according to concentrations of PCBs and DDTs in surrounding water and sediment (*17, 18*). As well, a significant spatial and seasonal pattern in exposure modeling for human health risk estimates for recreational fish consumers has been addressed (*18*). Consideration of spatial and temporal characteristics of contaminant bioaccumulation in fish reduced risk estimates as much as one order of magnitude lower than those obtained without the spatial and temporal consideration. In addition to improving our understanding of the chemical fate and transport in the environment, understanding and consideration of spatial and seasonal distribution of contaminants can reduce uncertainty associated with chemical exposure and provide a quantitative expression of the confidence in risk estimates. Consideration of spatial and seasonal variation can help support the decision-making process for risk assessment and risk management of contaminated water and sediment.

Spatial factors include sources of chemical contaminants, location of sensitive biological resources, routes of exposure, and factors that may modify contaminant mobility and availability (i.e., changing composition of sediment, abundance of microorganisms involved in contaminant biodegradation) (*19*). Temporal factors include seasonal changes in physical, chemical, or biological aspects of ecosystem that may impact the potential for exposure (*19*). The influence of seasonal variation on contaminant concentration and load may vary across a wide spectrum of the watershed characteristic. Foster et al. (*20*) reported increased water movement during storm flow enhanced PCB, organochlorine pesticides, and PAH riverine transport in both dissolved and particulate phase at the Susquenhanna River Basin, Maryland, USA. Soderstrom et al. (*21*) observed increased concentrations of DDTs and changes in DDD: DDE ratios (DDT metabolites) after snowmelt due to resuspension of surface sediment in a eutrophic and an oligotrophic lake. By contrast, PCB concentrations in the Eman River, Sweden were inversely related to river discharge due to dilution processes (22). In addition, seasonal variation in physical or chemical parameters (i.e., temperature or pH) can modify the bioavailability of contaminants and consequently change the nature of exposure (*19*).

Although the spatial and seasonal distributions of chemical contaminants such as PCBs and organochlorine pesticides have been widely studied, significant information gaps exist for their seasonal bioavailability. It is the freely dissolved fraction, not particulate fraction or dissolved fraction associated with dissolved organic matter, that is most bioavailable and relevant to the internal concentrations of the target organisms (*5*). Because PCBs and organochlorine pesticides are a problem of global concern, such information gaps must be addressed to promote a more complete understanding of their bioavailability characteristics that may be influenced by spatial and seasonal factors.

Passive sampling techniques as surrogates for evaluating bioavailability. Bioavailability is the key to determining the nature of the exposure and the toxicity of a chemical in the environment. Any chemical estimates of bioavailability must be correlated with biological measures of bioavailability or toxicological bioavailability (*5*). Measuring the actual dose or toxicant concentration at the target site is ideal for biologically effective dose and toxicity assessment (*5*). Alternatively, whole body internal concentrations are

sufficiently good approximations of target-site concentrations for toxic chemicals (*5*). However, measuring bioavailable chemicals in the exposed organisms in a field conditions sometimes fails to accurately reflect environmental contaminant concentrations because of residue metabolism and depuration and the effects of environmental stressors on organism health (*23*).

Recent developments in passive sampling devices provide an alternative to understand chemical bioavailability (*7, 24, 25*). These new developments in sampling technique fit well into chemical uptake and bioconcentration / bioaccumulation in certain aquatic organisms (*5, 15, 25-27*). Instead of measuring the total concentration in an environmental medium, the passive sampling technique measures the concentration in a reference phase which can be brought into equilibrium with the medium (*7, 28*). The availability of a chemical is measured based on the chemical potential which is logarithmically related to its fugacity and linearly related to its freely dissolved concentration in a particular medium (*7, 28*).

Figure 1.3 illustrates three uptake phases for a passive sampling device, which includes the generalized uptake profile according to Equation 1 – a first-order kinetic model (*7*):

$$C_{sampler}(t) = C_{medium} \bullet \frac{k_1}{k_2} \bullet (1 - e^{-k_2 \cdot t}) \quad (1)$$

where $C_{sampler}(t)$ is the concentration of the contaminant in the sampler as a function of time, t, C_{medium} is the concentration in the environmental medium, and k_1 and k_2 are the uptake rate and the elimination rate constants, respectively.

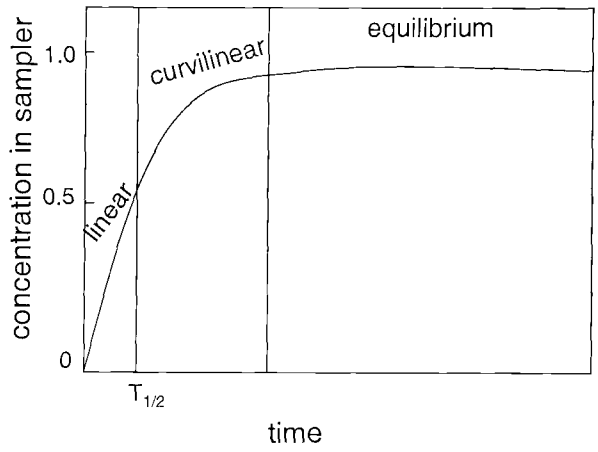


Figure 1.3 Generalized uptake profile for a passive sampling device. The passive sampling technique generally operates in three regimes: linear or kinetic, curvilinear or intermediate, and equilibrium.

Low density polyethylene membranes, typically filled with triolein, have been successfully deployed as passive sampling devices designed to accumulate nonpolar and moderately polar organic contaminants from water, sediments, and air (*15, 27, 29, 30*). This device is known as semipermeable membrane device (SPMD) introduced by Huckins et al. (*25*). It consists of polyethylene lay flat tubing containing a thin film of a neutral lipid, ≥ 95% pure triolein (1,2,3-tri[cis-9-octadecenoyl]glycerol) (*25*). The diameters of the transient cavities on polyethylene membrane range up to 10 Å, which effectively preclude sampling of any contaminant molecules associated with dissolved organic matter or particulates (*31*). It has been proposed that the SPMD mimics key mechanisms of bioconcentration including diffusing through biomembranes and partitioning between organism lipid and the surrounding medium , but without metabolism (*31*). For example, uptake of organochlorine contaminants was similar in SPMD, fish, and mussels (*25-27*). Determining dissolved/bioavailable concentrations with this technique would be a better measure to assess bioconcentration / bioaccumulation factors in aquatic organisms as compared with filtered water methodology, leading to a better prediction of environmental risk and risk assessment.

The three compartment model describing the mechanism for SPMD accumulation has been established (*28*). The model consists of three compartments; water, polyethylene membrane, and triolein, and three mass transfer step as described in Figure 1.4 by Gale (*28*).

Figure 1.4 shows a dissolved chemical is transported to the aqueous diffusion film via turbulent-diffusive transport through the aqueous film and partioning into polyethylene membrane, and diffusive transport through the polyethylene film, and partitioning between it and the triolein. It is noted that polyethylene as a chemical reservoir in SPMD is accounted for by the three compartment model and it is worth the effort to further develop it as an alternative passive sampling device as present in Chapter 3.

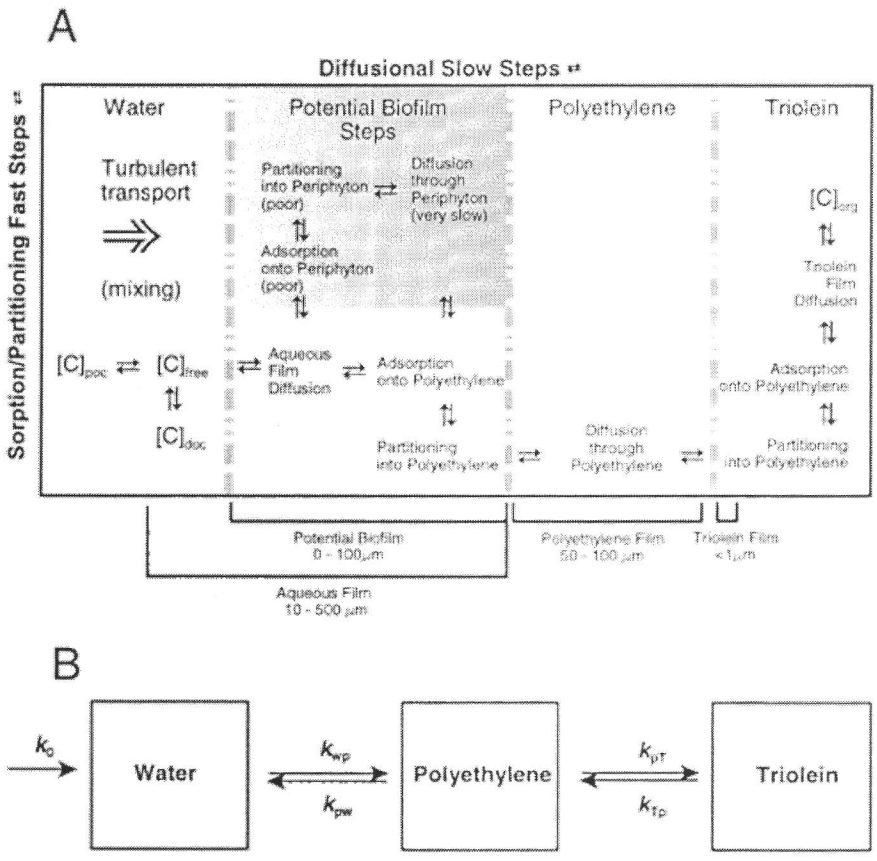


Figure 1.4 The three compartment model for contaminant accumulation by SPMD as proposed by Gale, 1998 (*28*). A) Proposed passive sampling steps for SPMD accumulation. B) The three compartment model consisting of water, polyethylene and triolein. Rate constants for input into the water (k_0),accumulative mass transfer from water to polyethylene (k_{wp}), from polyethylene to triolein (k_{pT}), and the corresponding clearance rate constants for mass transfer from water to polyethylene (k_{wp}) and from polyethylene to triolein (k_{pT}) are shown with their respective arrows.
(Figure is adapted from Gale, 1998)

The linear uptake model (Figure 1.3) is desired for the integrative sampling approach for SPMD application (*23*). Huckins et al. (*23*) have derived the first order kinetic model (Equation 1) to estimate bioavailable water concentrations from SPMD concentrations in the linear uptake phase and can be rewritten as

$$C_w = C_{SPMD} \bullet M_{SPMD} \bullet . R_s^{-1} \bullet . t^{-1} \quad (2)$$

where C_{SPMD} is the concentration of the bioavailable contaminant in the SPMD, M_{SPMD} is the mass of SPMD in grams R_s is the sampling rate in liters per day of a standard 1-g triolein SPMD, and t is the exposure time is days.

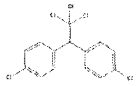
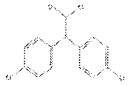
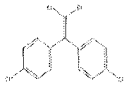
Using Equation (2) and available R_s values (*26, 32, 33*), the bioavailable water concentrations of hydrophobic contaminants can be estimated. For exposure conditions of low to moderate flow/turbulence, SPMD uptake is under membrane control for compounds with log $K_{ow} < 4.4$ and under aqueous boundary layer control for compounds with log $K_{ow} \geq 4.4$ (*23*). Thus the SPMD uptake of chemicals with log $K_{ow} \geq 4.4$, which include most PCB congeners, organochlorine pesticides, and PAHs, are sensitive to site hydrodynamics. SPMD uptake rates are also affected by temperature and biofouling (*34*). Booij et al. (*24*) and Huckins et al. (*34*) have developed the use of permeability / performance reference compound (PRC) to provide an overall correction factor for variations in SPMD uptake rates under field condition.

Organochlorine compounds in aquatic system. Polychlorinated biphenyls (PCBs) and organochlorine pesticides including p,p'-DDT and its metabolites (p,p'-DDD and p,p'-DDE) and dieldrin are industrial and agricultural compounds. These compounds have environmental significance because of their stability, toxicity, and tendency to accumulate in lipids of organisms (*35-37*). Their production and use have been banned since 1970s. The current major source of these compounds releases to air, water, and soil is from the cycling of their residues remaining in the environment from one medium to another. Atmospheric transport also plays an important role in long-range transport of organochlorines from their initial source (*38*).

Organochlorine environmental transport varies depending on their chemical and physical properties. Table 1.1 lists physicochemical properties of target PCBs and organochlorine pesticides in this dissertation. Organochlorines exist in diverse states in surface water. They can be freely dissolved in surface water or associated with dissolved or particulate matter depending on their chemical and physical properties (*35-37*). All of these forms can be transported by water or distributed throughout surface water. Their major transport is mainly via waterborne particles but in a river containing small amount of particles, their major transport can occur in the dissolved phase (*35-37*). In addition to ingestion of PCBs or DDTs contaminated in the aquatic food chain, aquatic organisms (e.g. fish, mollusk) directly uptake bioavailable form of these contaminants from surrounding water into their body by passive diffusion through semipermeable membranes such as gill, lining of the mouth, or gastrointestinal tract (*35-37, 39*). After uptake, organochlorine contaminants are transported to different compartments of the body including sites of action, metabolism, storage and excretion. The toxic effects of a contaminant can differ between species, strains, sex and age groups (*35, 36, 40*).

DDT is highly toxic to aquatic organisms (*35*). Early developmental stages of fish and aquatic invertebrate are more susceptible than adults to DDT (*35*). The acute toxicity of p,p'-DDT to both vertebrates and invertebrates is attributed mainly to action upon axonal Na^+ channels (*41*). Apart from the action upon Na^+ channels, it has been reported that p,p'-DDT can act upon the K^+ channels and inhibit certain ATPase (*41*). In fish, cellular respiration may be the main toxic target of DDT due to the inhibition of ATPase which can affect osmoregulation (*35, 41*). The effects of DDT on a range of physiological functions and behavioral development in fish have been reported (*35*). It has been reported that DDT can cause skeleton deformities by impairing developmental processes and bone formation in certain fish species (*42*). Pathologic changes by DDT are also observed in the liver and reproductive organs of laboratory animals (*35*).

Table 1.1 List of target PCBs and organochlorine pesticides and their physicochemical properties at 20-25 °C

chemical	chemical structure	water solubility (mg/L)[a]	Log K_{ow} [a]	sorption coefficient, log K_{oc} [a]	vapor pressure (P_a) [a]
- Trichlorobiphenyls					
PCB 37	3,4,4'	1.5×10^{-2}	5.9	4.8	4.5×10^{-3}
- Tetrachlorobiphenyls					
PCB 44	2,2',3,5'	1.0×10^{-1}	6.0	4.7	6.4×10^{-3}
PCB 49	2,2',4,5'	1.6×10^{-2}	6.1	5.7	7.4×10^{-3}
PCB 52	2,2',5,5'	3.0×10^{-2}	6.1	3.9	2.0×10^{-3}
PCB 60	2,3,4,4'	4.0×10^{-2}	6.3	5.7	2.2×10^{-3}
PCB 74	2,4,4',5	NA	6.7	NA	NA
PCB 77	3,3',4,4'	1.0×10^{-3}	6.5	4.4	2.0×10^{-3}
- Pentachlorobiphenyls					
PCB 87	2,2',3,4,5'	4.0×10^{-3}	6.5	5.7	2.3×10^{-3}
PCB 99	2,2',4,4',5	1.1×10^{-2}	6.6	5.7	1.5×10^{-3}
PCB 101	2,2,4,5,5'	1.0×10^{-2}	6.4	5.7	3.5×10^{-3}
PCB 105	2,3,3',4,4'	NA	6.0	NA	NA
PCB 114	2,3,4,4',5	NA	6.7	NA	NA
PCB 118	2,3',4,4',5	NA	7.1	NA	NA
PCB 126	3,3',4,4',5	NA	6.9	NA	NA
- Hexachlorobiphenyls					
PCB 128	2,2',3,3',4,4'	6.0×10^{-4}	7.0	5.3	3.4×10^{-4}
PCB 138	2,2',3,4,4',5'	1.5×10^{-3}	6.7	5.9	5.0×10^{-4}
PCB 153	2,2',4,4'5,5'	1.0×10^{-3}	6.9	5.9	7.0×10^{-4}
PCB 156	2,3,3',4,4',5	NA	7.2	NA	NA
PCB 166	2,3,4,4',5,6	NA	7.0	NA	NA
PCB 169	3,3',4,4',5,5'	5.1×10^{-4}	7.6	6.6	5.4×10^{-5}
- Heptachlorobiphenyls					
PCB 170	2,2',3,3',4,4',5	5.0×10^{-4}	7.1	5.6	1.3×10^{-5}
PCB 180	2,2',3,4,4',5,5'	3.1×10^{-4}	7.2	5.8	3.1×10^{-5}
PCB 183	2,2',3,4,4',5',6	NA	7.2	NA	NA
PCB 187	2,2',3,4',5,5',6	4.0×10^{-4}	7.2	5.5	9.4×10^{-5}
PCB 189	2,3,3',4,4',5,5'	NA	7.7	NA	NA
- Organochlorine pesticides					
p,p'-DDT		5.5×10^{-3}	5.7	6.3	2.5×10^{-5}
p,p'-DDD		2.0×10^{-2}	6.1	5.0	1.3×10^{-4}
p,p'-DDE		1×10^{-1}	6.0	4.7	8.7×10^{-4}
dieldrin		2×10^{-1}	3.5	4.1	4.0×10^{-4}

NA – not available

[a] Sources: Mackay et al. (*43*), Augustijn-Beckers et al. (*44*), Petty et al.(*45*), and Eisler and Belisle (*46*).

Dieldrin is highly toxic to aquatic crustaceans and fish (*36*). Several studies have revealed that dieldrin toxicity increases with increasing temperature (*36*). It has been reported that dieldrin can produce adverse enzymatic and hormonal change in fish that lead to impaired reproductive ability (*36*). Different life stages of fish have been found to have different susceptibility to dieldrin. Eggs were resistant and juvenile stages were less susceptible than adults (*36*).

Many studies have reported PCB toxicity in both in vitro and in vivo studies (*40*). The toxicity of individual PCBs is related to the molecular structure. The most potent congeners are those that contain non-ortho or mono-ortho chlorine substituents, so called co-planar PCBs, which have a similar structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. These dioxin-like PCBs act upon aryl hydrocarbon (Ah) receptors, with consequent induction of cytochrome P450 1A1/2 and Ah-receptor mediated toxicity (i.e. immunotoxicity, disruption of multiples endocrine pathways, developmental toxicity, reproductive toxicity, carcinogenicity, tumor promotion, hepatotoxicity) (*40*). Non-planar PCBs elicit toxic responses including neurobehavioral, neurotoxic, carcinogenic and endocrine changes by acting through multiple unrelated mechanisms (*47*).

Although the toxicology of PCBs has been studied extensively, the degree of toxicity and nature of the effects remain highly debatable. The complexity of PCB contamination with the possibility of interactive effects between different congeners and/or with other persistent contaminants have made ecological effects of PCBs difficult to prove (*41*). Recently, the influence of PCBs on the endocrine system has been a subject of particular environmental interest. Aquatic communities may be susceptible to endocrine disruption due to their association with PCB contaminated sediments and surface water. Many studies have suggested PCBs adversely affected gonadal development, vitellogenin expression, thyroid function and sexual differentiation in aquatic organisms (*37, 40, 46, 48*). It has been suggested that the decline in certain fish populations may be associated with developmental and reproductive impairment caused by PCBs (*48*).

Study area: the lower Willamette River at Portland Harbor, Oregon. The Willamette River in western Oregon is one of only 14 American Heritage Designated Rivers

(*49*). The river has the thirteen the largest stream flow in the United States and yields more runoff per square mile than any other large river in the US (*50*). The Willamette River flows approximately 187 miles northward through Portland, Oregon's largest metropolitan area, before joining the Columbia River. After this joining, the Columbia River flows an additional 100 miles westward to the Pacific Ocean. The Willamette River provides a significant migratory corridor, nursery habitat and adult forage for runs of salmon, and nearly 50 species of fish have been identified in the river (*51*). Recreational and sport fishing are extremely popular throughout the lower Willamette basin. Most development along the Willamette River has been located within the Portland Harbor. Portland Harbor is heavily industrialized, and contains a multitude of facilities and both private and municipal waste water outfalls. The harbor generally has a low sediment transport capacity (*8*). Surface water runoffs are determined by climate influence and vary according to season (*8*).

The presence of PCBs, pesticides, PAHs, dioxins/furans, arsenic, cadmium, chromium, copper, lead, mercury, zinc, organotins, pentachlorophenol, and solvents has been demonstrated in Portland Harbor sediment within a six-mile stretch from the southern tip of Sauvie Island to Swan Island (RM 3.5 –RM 9.5)(*8*). These contaminants may have entered the river via spillage during product shipping and handling, direct disposal or discharge, accidental spills, contaminated ground water discharge, surface water runoff, stormwater discharge, or contaminated soil erosion (*52*). These contaminants can pose risks to people, fish and other wildlife and the detected levels were high enough to place Portland Harbor on the Federal National Priority List (NPL, commonly known as superfund) (*52*). The NPL is the US Environmental Protection Agency (EPA)'s list of the national most contaminated hazardous waste sites which may pose dangers to public health and the environment and are therefore targeted for cleanup (*9*). Portland Harbor became a superfund site on December 1, 2000 (*9*). The area covers the six-mile stretch between the southern tip of Sauvie Island to Swan Island (RM 3.5 –RM 9.5) and contains McCormick and Baxter Creosoting Co. (Portland plant) superfund site.

Our previous study found PCB and DDT residues accumulated in three recreational fish species from the Portland Harbor exceeded the US EPA fish advisory's screening values and potentially posed adverse health effects to the recreational and subsistence fishers (*53,*

54). Bioaccumulation of PCBs and DDTs in fish is related to the concentrations of PCBs and DDTs in surrounding water and sediment and can change seasonally (*17, 18*). Consequently, the seasonal pattern in exposure modeling for environmental and human health risk assessment can affect the accuracy of risk estimates (*18*). As surface water runoffs within the harbor are determined by climate influence and vary according to season (*8*), PCB and DDT river concentrations, particularly the bioavailable concentrations may change seasonally and consequently change the nature of exposure. This can affect both aquatic organisms and human health. The river characteristics make Portland Harbor an excellent candidate for studying the seasonal bioavailability of PCBs and organochlorine pesticides in surface water.

Hypotheses and objectives

Hypothesis 1. The water concentrations of bioavailable organochlorines including PCBs, p,p'-DDT and its metabolites (p,p'-DDD and p,p'-DDE), and dieldrin change seasonally (Chapter 2).

Objectives

- To determine the bioavailable concentrations of organochlorines in surface water using passive sampling device
- To evaluate the spatial influence on the distribution of bioavailable organochlorines in surface water
- To evaluate the seasonal distribution of bioavailable organochlorines in surface water and to assess the potential factors influencing their seasonal concentrations

Hypothesis 2. Seasonal changes affect the nature of exposure to organochlorines in surface water by aquatic organisms and local fishers who consume fish in the study area (Chapter 2).

Objective

- To screen the potential impacts associated with seasonal changes on aquatic organisms and local fish consumers by comparing seasonal concentrations of bioavailable organochlorines to the national recommended water quality criteria (*10*) and the Oregon water quality criteria (*11*)

Hypothesis 3. The solvent- free low density polyethylene membrane lay flat tubing (LFT) is as efficient as the widely used passive sampling device in sampling bioavailable hydrophobic organic contaminants in surface water (Chapter 3).

Objectives

- To evaluate the use of LFT as an alternative device for monitoring bioavailable hydrophobic organic contaminants in surface water
- To compare the efficiency of LFT with the semipermeable membrane device (SPMD)

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CHAPTER 2

EVALUATION OF SEASONAL BIOAVAILABILITY OF ORGANOCHLORINE PESTICIDES AND PCBs AT A SUPERFUND SITE USING PASSIVE SAMPLING DEVICES

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Abstract

Although the spatial and seasonal distribution of polychlorinated biphenyls (PCBs) and organochlorine pesticides (i.e., DDT, dieldrin) have been widely studied, significant information gaps exist for their bioavailability. Because PCBs and organochlorine pesticides are a problem of global concern, such information gaps must be addressed to promote a more complete understanding of their bioavailability characteristics that may influence spatial and seasonal factors. The present study focused on the spatial and seasonal distribution of bioavailable organochlorine pesticides and PCBs within the surface water of a contaminated harbor. Passive sampling devices were intensively deployed just outside the shipping channel adjacent to various land uses on the lower Willamette River, Oregon including Portland Harbor superfund site in summer and fall during 2001-2004. An increase of estimated bioavailable ΣDDTs (sum of p, p'-DDT, p, p'-DDD, and p,p'-DDE) concentrations was strongly affected by the local historic productions of DDTs and seasonal changes in river conditions. The dominance of bioavailable p, p'-DDD and high DDD/DDE ratios observed during periods of low flow in summer suggested direct inputs of p, p'-DDD and conditions favoring reductive dechlorination of p, p'-DDT to p, p'-DDD. The estimated bioavailable concentrations and daily loads of PCBs and dieldrin increased during periods of high precipitation, high river flow in fall, especially during episodic rainstorms. The similarity of PCB congener profiles and PCB homolog ratios at the industrial area and at the urban/residential areas suggested the local sources of PCBs at the industrial area were important enough to significantly increase the concentrations of bioavailable PCBs in surface water but not so substantial as to change composition relative to surface water inputs from upstream. Bioavailable concentrations organochlorine compounds seasonally exceeded the national and the Oregon water quality criteria. These seasonal exceedances suggest the potential impacts associated with seasonal changes of bioavailable organochlorine distributions in surface waters and the significances of considering realistic seasonal and site-specific conditions in risk assessment and water quality management.

Keywords –

Willamette River bioavailability organochlorines contaminants seasonal influence

Introduction

The freely dissolved form of hydrophobic organic contaminants is considered environmentally relevant to their bioavailability, toxicity, mobility and degradation processes (*1-3*). It is the freely dissolved form of the contaminant which is transported across biological membranes of aquatic organisms and potentially exerts toxic effects (*2-4*). A reduction in the freely dissolved concentration of the contaminant refers directly to reduced bioavailability, and vice versa. However, there is no clear distinction between the process that controls the distribution of chemical contaminants in an environment and those that directly affect bioavailability (*5*). Spatial and temporal factors including seasonal changes in physical, chemical and biological aspects of the aquatic ecosystem may modify contaminant bioavailability and this changes concentration available for uptake from water by aquatic organisms. The potential uptake of toxic contaminants is affected by changes in either the concentrations of the contaminant in the organism's immediate environment or the environmental bioavailability of the contaminant (*5*). Despite the importance of environmental bioavailability, very few studies have actually evaluated the distribution of chemical bioavailability and the influencing factors in the field under different and often more variable geochemical or physiological conditions than in the laboratory.

A consideration of the spatial and seasonal aspects is essential to chemical exposure in ecological risk assessment (*6*). These variables affect the concentrations available for uptake from surrounding media by organisms. Several studies have indicated bioaccumulation of polychlorinated biphenyls (PCBs) and DDTs (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane and its metabolites) in fish and lower trophic level biota seasonally changed according to concentrations of PCBs and DDTs in surrounding water and sediment (*7, 8*). As well, a significant spatial and seasonal pattern in exposure modeling for human health risk estimates for recreational fish consumers has been addressed (*8*). Consideration of spatial and temporal characteristics of contaminant bioaccumulation in fish reduced risk estimates as much as 1 order of magnitude lower than those obtained without the spatial and temporal consideration. In addition to improving our understanding of chemical fate and transport in the environment, understanding and consideration of spatial and seasonal distribution of contaminants can reduce uncertainty associated with chemical exposure and

provide a quantitative expression of the confidence in risk estimates. Consideration of spatial and seasonal variation can help support the decision-making process for risk assessment and risk management of contaminated water and sediment.

Spatial factors include sources of chemical contaminants, location of sensitive biological resources, routes of exposure, and factors that may modify contaminant mobility and availability (*9*). Temporal factors includes seasonal changes in physical, chemical, or biological aspects of ecosystem that may be a potential for exposure (*9*). The influence of seasonal variation on contaminant concentration and load may vary across a wide spectrum of watershed characteristic. Foster et al. (*10*) reported increased water movement during storm flow enhanced transport of PCBs, organochlorine pesticides, and polycyclic aromatic hydrocarbons (PAHs) in both dissolved and particulate phase at the Susquenhanna River Basin, Maryland, USA. Soderstrom et al. (*11*) observed increased concentrations of DDTs and changes in DDD: DDE ratios after snowmelt due to resuspension of surface sediment in a eutrophic and an oligotrophic lake. By contrast, PCB concentrations in the Eman River, Sweden were inversely related to river discharge due to dilution process (*12*). In addition, seasonal variation in physical or chemical parameters (i.e., temperature or pH) can modify the bioavailability of contaminants and consequently change the nature of exposure (*9*). Although the spatial and seasonal distributions of chemical contaminants such as PCBs and DDTs have been widely studied, significant information gaps exist for their seasonal bioavailability. Because PCBs and organochlorine pesticides are a problem of global concern, such information gaps must be addressed to promote a more complete understanding of their bioavailability characteristics that may be influenced by spatial and seasonal factors.

The Willamette River in western Oregon is one of only 14 American Heritage Designated Rivers (*13*). The river has the thirteenth largest stream flow in the US and yields more runoff per square mile than any other large river in the US (*14*). Recreational and sport fishing are extremely popular throughout the lower Willamette basin. The lower end of the Willamette River, Portland Harbor, is heavily industrialized and contains a multitude of facilities and both private and municipal waste water outfalls. The lower Willamette River at Portland Harbor is deep, slow moving, and tidally influenced (*15*). Due to elevated

concentrations of PCBs, organochlorine pesticides, dioxins/furans, PAHs, and heavy metals in the harbor sediment, Portland Harbor has been placed in the Federal National Priority List (NPL), commonly known as superfund (*16*).

Our previous study found PCB and DDT residues accumulated in three recreational fish species from the Portland Harbor exceeded the US EPA fish advisory's screening values and potentially posed adverse health effects to the recreational and subsistence fishers (*17, 18*). Bioaccumulation of PCBs and DDTs in fish is related to the concentrations of PCBs and DDTs in surrounding water and sediment and can change seasonally (*7, 8*). As surface water runoffs within the harbor are determined by climate influence and vary according to season (*15*), PCB and DDT river concentrations, particularly the bioavailable concentrations may change seasonally and consequently change the nature of exposure. The consequence can affect both aquatic organisms and human health. The river characteristics make Portland Harbor an ideal study area for evaluating seasonal bioavailability of PCBs and organochlorine pesticides in surface water.

The ultimate goals of this study were to understand the seasonal distribution of bioavailable PCBs, p,p'-DDT and its derivatives, and dieldrin in surface water and to evaluate the potential environmental factors influencing their bioavailability. The passive sampling technique using semipermeable membrane device (SPMD) (*19, 20*) was chosen to get a time-integrated measure of their bioavailable fraction. The SPMD consists of polyethylene lay flat tubing containing a thin film of a neutral lipid, ≥ 95% pure triolein (1,2,3-tri[cis-9-octadecenoyl]glycerol) (*21*). It has been proposed that SPMD mimics key mechanisms of bioconcentration including diffusing through biomembranes and partitioning between organism lipid and the surrounding medium , but without metabolism (*19*). In this work, the influences of seasonal and episodic events coupled with *in situ* water chemistry on their bioavailable transport were intensively investigated. The potential environmental factors influencing their bioavailability were discussed. To screen the potential impacts associated with seasonal changes on aquatic organisms, the bioavailable concentrations were compared to the national recommended water quality criteria (*22*) and the Oregon water quality criteria (*23*).

Materials and Methods

Materials and chemicals. Standard SPMDs were purchased from Environmental Sampling Technologies (EST, St. Joseph, MO). Standards of organochlorine pesticides (purities ≥ 98.5%) and PCBs (purities ≥ 99%) were from Chem Service, Inc. (West Chester, PA) and AccuStandard (New Haven, CT), respectively. All solvents used were pesticide or Optima® grade from Fisher Scientific (Fairlawn, NJ). Certified reference material of PCB congeners and organochlorine pesticides in cod liver oil (SRM 1588a) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). SRM 1588a was used for method validity and accuracy purposes for the determination of PCBs and organochlorine pesticides in complex lipophilic matrices such as triolein.

Selection of 25 target PCB congeners were based on their toxicity, frequency of occurrence, and abundance in environmental matrices (*24*). Target PCB congeners included dioxin-like congeners (tetra-CB; PCB 77, penta-CB; PCB 105, PCB 114, PCB 118, PCB 126; hexa-CB; PCB 156, PCB 169; hepta-CB; PCB 189) and non dioxin-like congeners (tri-CB; PCB 37, tetra-CB; PCB 44, PCB 49, PCB 52, PCB 60, PCB 74, penta-CB; PCB 87, PCB 99, PCB 101, hexa-CB; PCB 128, PCB 138, PCB 153, PCB 166, hepta-CB; PCB 170, PCB 180, PCB 183, PCB 187). Target organochlorine pesticides were p,p'-DDT, p,p'-DDD, p,p'DDE, and dieldrin.

Study area. The sampling areas were located at the lower Willamette River at Portland Harbor from River Mile 1 to 18 and contained the Portland Harbor superfund site (River Mile 3.5 to 9.5) and the McCormick and Baxter Creosoting Co. (Portland plant) superfund site (River Mile 7 East). The sampling sites were outside the main shipping channel and often near stream outlets of various upstream land uses. The eleven sampling sites (Figure 1) were at River Mile 1 East (Columbia Slough; industrial area), River Mile 3.5 West (Sauvie Island at Multnomah Channel; industrial and urban area), River Mile 3.5 East (opposite to Multnomah Channel; industrial area) , River Mile 7 West (Railroad Bridge; industrial area), River Mile 7 East (McCormick and Baxter superfund site), River Mile 8 East (Swan Island; industrial area), River Mile 12 East (under Steel Bridge/ Interstate 5 Highway; downtown/urban area), River Mile 13 West (Hawthorne Bridge; downtown/urban area),

River Mile 15 East (Ross Island; a sand and gravel operation/ undeveloped area), River Mile 17 East (golf course, park), and River Mile 18 West (Mouth of Johnson Creek; urban/agricultural creek, residential area).

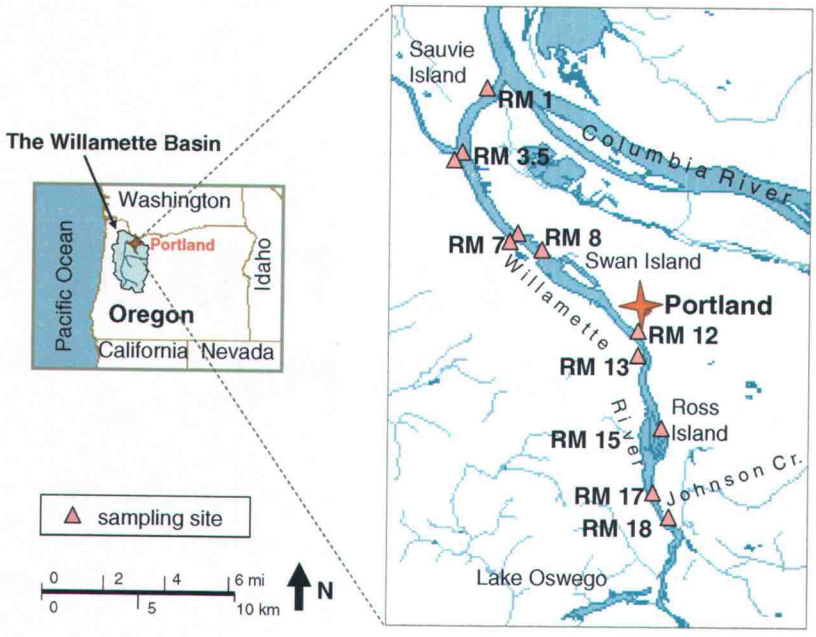

Figure 2.1 The lower Willamette River at Portland, Oregon, showing locations of the sampling sites (RM = river mile)

Sources of precipitation and stream flow data. Precipitation and stream flow data were needed to characterize the river conditions during sample collection. Precipitation data were obtained from the National Weather Service Forecast Office – Portland, OR (*25*). The station was located at the Portland International Airport which was 9 miles northeast of downtown Portland. Stream flow data recorded at River Mile 12.8 were obtained from the US Geological Survey (*26*). Stream flow data were also used to convert estimated surface water concentrations using SPMD into loading estimates. A daily mass load was calculated by multiplying the estimated river concentration using SPMD by the average daily flow rate through the river.

Sample collection. Five individual SPMDs were loaded in a flow-through stainless steel cage. Each cage was submerged approximately 10 ft from the river bottom and was suspended with "anchor-cable-cage-cable-float" arrangement. The river depth at sampling locations ranged from 2-50 ft. At the sampling sites at RM 7 East where the river was generally less than 10 ft. deep, the samplers were suspended below water surface but above the sediment. SPMDs were deployed for 7-21 days. Water temperature and other water chemistry parameters (dissolved oxygen, specific conductivity, oxidation-reduction potential, pH, NH_4^+-N, and NO_3^- -N concentrations) were measured during deployment and retrieval using an YSI 6920 SONDE (Yellow Springs, OH). The average temperature ranged from 9 to 22 °C. Water samples were also collected for the analysis of total organic carbon (TOC), dissolved organic carbon (DOC), total suspended solids (TSS), and total dissolved solids (TDS).

On retrieval, SPMDs were cleaned by gently rubbing with gloved hands in on-site water, then rinsed in 1N HCl (Trace metal grade, Fisher Chemical, Fairlawn, NJ), 18 MΩ·cm water, acetone (Pesticide grade, Fisher Scientific, Fairlawn, NJ), and isopropanol (Pesticide grade, Fisher Scientific, Fairlawn, NJ), respectively. Cleaned SPMDs were kept in clean glass amber jars and transported on ice-packs. Samples were stored at -20 °C until analysis.

Sample extraction and chemical analysis. SPMD extraction and cleanup were based on established protocols and only modified slightly as necessary (*19, 27*). SPMDs were dialyzed in hexanes, 400 mL for 5 SPMDs (Pesticide grade, Fisher Scientific, Fairlawn, NJ) for 18 hr, followed by a second dialysis with fresh hexanes for 6 hr. The combined dialysates were concentrated by rotary evaporation and a TurboVap® LV evaporator (Zymark®, Hopkinton, MA). The samples were cleaned and fractionated using gel permeation chromatography (Waters® gel permeation chromatography cleanup system; Water® 515 HPLC pump, 717 Plus autosampler, 2487 dual λ absorbance detector, and fraction collector II, Milford, MA) with dichloromethane (Optima®, Fisher Scientific, Fairlawn, NJ) as the mobile phase at the flow rate of 5 mL/min. Appropriate fractions were determined by analyzing standards and fortified samples. The appropriate fraction (14-20 min) was collected and split into two equal sub-fractions: 1) PCB and organochlorine pesticides for this study 2) PAHs for another study. PCB and organochlorine pesticide fraction was subjected to volume

reduction using a TurboVap® LV evaporator and solvent exchanged into iso-octane (Pesticide grade, Fisher Scientific, Fairlawn, NJ) with a final volume of 1 mL.

PCB congeners and OC pesticides were determined using GC-ECD (Agilent Technologies 6890N Network GC system, Palo Alto, CA) dual capillary columns (db-xlb and db-17 with length 30 m, i.d. 0.25 mm, and film thickness 0.25 μm, J&W Scientific Inc., Agilent Technologies, Palo Alto, CA) /dual detectors. Injector and detector temperature were at 250 °C, and 350 °C, respectively. The GC system was operated with helium carrier gas and nitrogen makeup gas. The oven temperature program was as follows: 100 °C (1 min) and increased at 1.2 $°Cmin^{-1}$ to 265 °C (2 min).

Field duplicates and blanks (trip blank, field blanks, and field extraction blanks) accompanying the deployed passive sampling devices during deployment, retrieval, and transportation to the laboratory were included in every batch. These field blanks and laboratory control blanks (i.e., procedural blanks, laboratory SPMD controls, fortified samples, and standard reference material) represented 30 to 40% of a sample set and were processed and analyzed exactly as the deployed samples. The method detection limits (MDL) were determined as three times the heights of coincident peaks observed for each compound in the laboratory SPMD control. For those analytes having no coincident peak, the MDLs were set at a value equivalent to the lowest standard in the respective calibration curve.

Because contaminant uptake rates by SPMDs can change due to changes in temperature, flow velocity of the surrounding water and buildup of periphyton on the membrane surface, an *in situ* measurement to account for these field variables is needed (*28, 29*). PCB 8, PCB 82 and endrin were used as permeability/performance reference compounds (PRCs) to measure the overall variations in SPMD uptake rates under field conditions. PRCs are analytically non-interfering compounds with moderate to relatively high fugacity from SPMDs and have physico-chemical properties similar to the target analytes, which are added to the SPMDs prior to sealing the membrane tubes (*29*). Because both the uptake and the dissipation of organic chemicals are controlled by the same molecular processes, *in situ* dissipation rates of PRCs would allow for estimating *in situ* SPMD uptake rates under

various field conditions (*28, 29*). The PRC approach has been well described by Booij et al. (*28*) and Huckins et al.(*29*).

None of the target analytes were identified in field blanks and laboratory control blanks. The average percent recoveries of fortified samples were as follows: PCBs 69 ± 43%, p,p'-DDT 71 ± 27%, p,p'-DDD 80 ± 21%, p,p'-DDE 66 ±16%, and dieldrin 73 ± 18%. The average percent recoveries in certified reference material were as follows: PCBs 94 ± 26%, p,p'-DDT 98 ± 18%, p,p'-DDD 105 ±17%, p,p'-DDE 76 ± 16% and deildrin 89 ± 6%.

Data analysis. The basic theory and mathematical models required for estimation of analyte water concentrations from the concentrations in the SPMD have been described by Huckins et al. (*20*). The average percent recoveries of PRCs in the deployed SPMDs indicated the linear uptake model could be assumed for all target analytes in this study. Differences in exposure temperature were corrected by using established SPMD sampling rates (R_s) for PCBs and organochlorine pesticides at multiple temperatures (*30-32*). The small variations of PRC dissipation rates among sampling sites and under different field seasons suggested the effects of membrane biofouling and flow velocity-turbulence at the membrane surface were considered negligible.

Estimates of water concentrations (C_W) from SPMD concentrations were calculated by the following equation (*20*),

$$C_W = C_{SPMD} \bullet M_{SPMD} \bullet R_s^{-1} \bullet t^{-1}$$

where C_{SPMD} is the concentration of the individual analyte in the SPMD, M_{SPMD} is the mass of SPMD in grams, M_{SPMD} is the sampling rate of a standard 1-g triolein SPMD, and t is the time in days.

Data interpretation was performed using SPSS® Version 10.0.1(SPSS Inc., 1989-1999), Sigma Plot 2002 for Windows Version 8.0 (SPSS Inc., 1986-2001), and Microsoft® Office Excel 2003 (Microsoft Corporation, 1985-2003). Standard descriptive statistics, two sample t-test and linear regression techniques were used to examine seasonal bioavailable

organochlorine concentrations and influence variables. Statistical analyses were considered significant at $p \leq 0.05$.

Estimates of bioavailable concentrations in surface waters were compared to the national recommended water quality criteria (*22*) and the Oregon water quality criteria (*23*). These guidelines were used as a screening tool to assess potential impacts associated with seasonal or episodic changes of contaminant distributions in surface waters. Fresh water aquatic life criteria are to protect the aquatic communities from toxic effects resulting from chronic exposure to toxic contaminants in water (*22*). This criterion concentration is an estimate of the highest concentration in surface water to which an aquatic community can be exposed without resulting in an unacceptable effect. Human health water quality criteria are to protect local fishers based on a 10^{-6} increased lifetime cancer risk from consumption of fish and shellfish from the concerning areas(*22*).

Results and Discussions

River conditions and water chemistry. The lower Willamette River at Portland Harbor could be typically characterized by seasonally defined conditions. We categorized the river conditions according to the average river flow, total precipitation, and average temperature during sample deployments. Over the sample deployment interval from 2001 to 2004, the river was categorized into low flow/low precipitation in summer (average river flow < 10,000 ft^3/s, total precipitation 0-1 in., average temperature ≥ 20 °C) and high flow/high precipitation in fall (average river flow > 10,000 ft^3/s, total precipitation > 1 in., average temperature < 20 °C). In addition, any intermittent precipitation or high river flow that occurred during sample deployment was considered as an episodic event in the present study.

Table 2.1 Summary of river conditions and water chemistry parameters at Portland Harbor during SPMD deployment.

sampling event	river flow (ft^3/s)	total rain (in.)	water temp. (°C)	pH	specific conductivity (mS/cm)	DO (mg/L)	ORP (mV)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)
08/02/01-	7000	0	22	7.4	0.12	7.2	140	0.054	2.1
08/16/01	(170)		(0.57)	(0.15)	(0.022)	(0.44)	(24)	(0.097)	(0.24)
09/13/01-	7200	0	20	7.4	0.11	8.6	210	0.12	2.2
09/20/01	(130)		(0.19)	(0.17)	(0.022)	(0.43)	(23)	(0.030)	(0.19)
10/16/01-	13000	2.93	13	7.4	0.096	11	200	0.14	1.4
11/06/01	(4000)		(0.40)	(0.13)	(0.034)	(0.15)	(25)	(0.046)	(0.24)
11/06/01-	27000	3.86	10	7.4	0.12	12	210	0.14	1.7
11/26/01[b,c]	(19000)		(0.14)	(0.066)	(0.027)	(0.41)	(14)	(0.024)	(0.18)
07/17/02-	8800	0.04	22	7.4	0.17	8.7	160	0.25	2.1
08/07/02	(190)		(0.37)	(0.14)	(0.19)	(0.63)	(22)	(0.14)	(0.66)
08/07/02-	8400	0	22	7.3	0.19	8.3	150	0.25	2.0
08/21/02	(180)		(0.35)	(0.17)	(0.25)	(0.63)	(29)	(0.18)	(0.70)
09/11/02-	9600	0.41	19	7.4	0.098	9.3	160	0.21	1.6
09/25/02	(400)		(0.76)	(0.079)	(0.015)	(0.44)	(28)	(0.021)	(0.49)
11/07/02-	11000	1.88	9.5	7.2	0.078	12	160	0.18	1.3
11/26/02	(2000)		(0.46)	(0.11)	(0.016)	(0.17)	(12)	(0.017)	(0.40)
10/01/03-	10000	2.32	18	7.2	0.10	10	280	0.24	0.62
10/15/03[b,c]	(620)		(0.22)	(0.18)	(0.017)	(0.86)	(23)	(0.034)	(0.094)
11/05/03-	14000	2.71	9.0	NA	NA	NA	NA	NA	NA
11/24/03[c]	(4800)		(1.0)						
07/08/04-	8300	0.04	23	7.2	0.097	9.5	160	0.032	0.75
07/29/04[b]	(390)		(0.85)	(0.10)	(0.011)	(1.2)	(28)	(0.017)	(0.18)
08/19/04-	10000	2.35	22	7.2	0.10	8.5	170	0.16	0.57
09/09/04[b,c]	(1700)		(0.26)	(0.051)	(0.018)	(1.2)	(28)	(0.028)	(0.12)
10/19/04-	21000	2.00	9.6	6.4[a]	0.078	12	310	0.17	0.16
11/09/04[c]	(4700)		(1.0)	(0.13)	(0.018)	(0.49)	(127)	(0.045)	(0.10)

values are average of 11 sampling sites; numbers in parenthesis are 1 SD; NA – data not available
[a] lower pH was measured at River Mile 3.5 East (pH = 4.2) and RM Mile 7 West (pH = 5.1). There was no overt failure of the probe. These two measurements appeared to be an outlier and we did not include to the average; [b] sewage overflow occurred before or during sample deployment; [c] rainstorm during sample deployment

Table 2.2 Summary results of total organic carbon (TOC), dissolved organic carbon (DOC), total suspended solids (TSS), and total dissolved solids (TDS) at the Willamette River, Oregon during SPMD deployments.

sampling date	TOC (mg/L)	DOC (mg/L)	TSS (mg/L)	TDS (mg/L)
11/27/01	2.4 (0.47)	NA	NA	NA
11/26/02	2.2 (0.81)	2.1 (0.83)	NA	NA
10/01/03	1.4 (0.0058)	1.3 (0.044)	NA	NA
11/05/03	1.6 (0.058)	1.6 (0.050)	4.2 (1.2)	120 (24)
07/08/04	1.7 (0.21)	1.8 (0.27)	6.8 (1.3)	66 (9.3)
07/29/04	1.9 (0.21)	1.9 (0.17)	7.8 (2.6)	94 (30)
08/19/04	1.7 (0.073)	1.8 (0.093)	7.8 (6.5)	86 (9.0)
09/09/04	1.8 (0.076)	1.7 (0.060)	6.8 (3.4)	74 (9.7)

- Values are averages of 10 sampling sites (River Mile 1 – 18) in 2001, and 4 sampling sites (River Mile 1, River Mile 7 West, River Mile 7 East, and River Mile 18) in 2002, 2003, and 2004. Spatial differences between sampling sites were not statistically significant, therefore the data were averaged.
- Numbers in parenthesis are 1 SD
- NA; data not available

The river characteristics and water chemistry during passive sampling device deployments are summarized in Table 2.1. *In situ* river physico-chemical parameters were collected at the river bottom, at 10 ft. from the river bottom where the samplers were located, and at the river surface. At each sampling site, water chemistry parameters of the three vertical levels were not statistically different within a sampling event. Also within the study area (18 miles), there was no statistical difference between the sampling sites. The physico-chemical properties measured at 10 ft. from the river bottom at the eleven sampling sites were therefore averaged and reported in Table 2.1. The difference in water temperature in summer and fall was approximately 10 °C. Dissolved oxygen concentrations ranged from 7.2 mg/L in summer to 12 mg/L in fall. The river pH was neutral. The oxidation-reduction potential tended to decrease in periods of low flow in summer ($p = 0.054$, *t*-test). There was no seasonal pattern observed for the river specific conductivity, NH_4^+-N, and NO_3^--N

concentrations. In addition, the concentrations of TOC, DOC, TSS, and TDS did not change seasonally (Table 2.2) which suggested that a major transport of contaminants in the lower Willamette River was in the aqueous phase. The study of aqueous phase bioavailable contaminant distribution in the present study is therefore relevant to temporal river contaminant characterization.

Distributions of bioavailable ΣDDT concentrations and potential sources.

Bioavailable ΣDDT concentrations (sum of p, p'-DDT, p, p'-DDD, and p, p'-DDE; below detection limits were treated as zero) from the eleven sampling sites over the four-year study period varied from 27 pg/L to 1500 pg/L with an average concentration of 190 pg/L. The highest concentrations were always observed at River Mile 7 West (350-1,500 pg/L). The concentrations of ΣDDTs in the Willamette River at River Mile 7 West were generally higher than dissolved ΣDDT concentrations of several other large rivers in the US including the San Joaquin River, California (< 1,000 pg/L) (*33*), the Missouri River, Missouri (27-270 pg/L) (*34*), and the Susquehanna River, Maryland (280 pg/L) (*10*). However they were comparable to or much lower than the concentrations in several rivers in other parts of the world such as the Humber catchments, UK; 220 – 1,800 pg/L(*35*), the Minjiang River, China; 40,000-230,000 pg/L (*36*) and the Kafue River, Zambia; 7,000-35,000 pg/L (*37*). It is important to note that only the studies at the Missouri River and the Kafue River used passive sampling devices to study the dissolved concentrations while the other studies used conventional filtered water methodology. The two approaches are not directly comparable since, unlike the passive sampling technique, the dissolved concentrations in filtered waters would include DDTs associated with dissolved organic matter. Therefore the non-passive sampling device technique may overestimate the truly dissolved bioavailable concentrations. However, in general, the Willamette River at Portland Harbor, particularly at River Mile 7 West is more contaminated with DDTs than many studied compromised rivers in the US, but it is generally less than compromised rives worldwide.

Figure 2.2 shows the average concentrations of ΣDDTs estimated from ΣDDT concentrations in the SPMD. Spatial distribution of ΣDDTs was observed both in summer and fall conditions. Bioavailable concentrations of ΣDDTs and each individual p, p'-homolog

at River Mile 7 West were significantly higher than any other sampling sites in both summer and fall ($p < 0.001$; ANOVA F-test and Tukey's HSD). The concentrations of bioavailable Σ DDTs downstream of River Mile 7 west were apparently diluted from the concentrations measured at River Mile 7 West. The average bioavailable ΣDDT concentrations at the upstream sampling sites including River Mile 7 East were approximately 10-fold less than those at River Mile 7 West (Figure 2.2).

DDT production and use in the US has been banned for three decades. Surface water contamination would remain only if DDTs are continuously introduced through atmospheric deposition, stream runoff, and non point source inputs of surrounding soils and sediment from both in and adjacent to the water body. A contribution of ΣDDTs from atmospheric deposition is not likely to account for a particular site-specific contamination within the 18-mile stretch. The 10-fold increases of bioavailable ΣDDTs in surface water at River Mile 7 West, as demonstrated in Figure 2.2, indicated the upstream sources were not as significant as the local source at River Mile 7 West. Our findings (Figure 2.2) combined with lack of organochlorine insecticides, including p,p'-DDE detected in the filtered water samples from the Willamette River tributaries by Anderson et al. (*38*) suggest the tributary influences on the bioavailable ΣDDT distributions in the Lower Willamette River are probably small. Data from Figure 2.2 further reveals that the ability of stream flow to transport bioavailable DDT and its metabolites during high flow periods in fall is insignificant. These findings indicate that sources upstream of the harbor are negligible for bioavailable ΣDDTs.

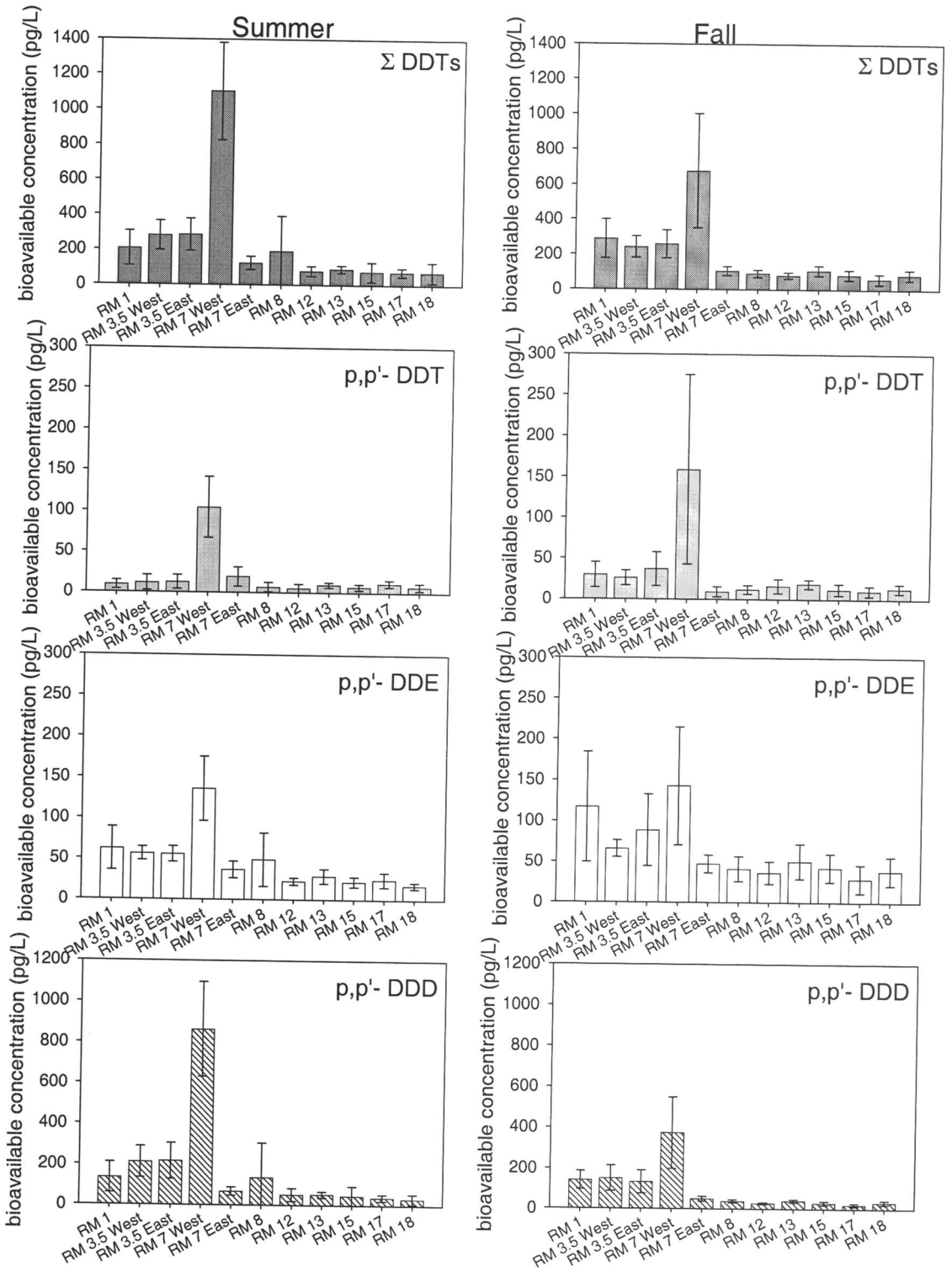

Figure 2.2 Average concentrations of bioavailable ΣDDTs in surface water at the lower Willamette River at Portland Harbor, Oregon, using passive sampling device (n=186). Passive sampling devices were deployed for 7-21 days during the periods of low flow in summer, and high flow in fall of 2001-2004.

The bioavailable ΣDDT concentrations at River Mile 7 West increased significantly during low-flow in summer sampling events, indicating a source of DDT in this area may be important. A former DDT manufacturing and handling facilities were approximately 1 mile upstream of the sampling site at River Mile 7 West. The spatial distribution of bioavailable Σ DDT concentrations coincided with the ΣDDT distributions found in sediment (*15*). Weston et al. (*15*) reported surface sediment samples collected within 1 mile downstream of the former DDT facilities contained ΣDDT concentrations approximately 4- to 10-fold higher than in the samples upstream or downstream. Historically discharged ΣDDTs in sediment could be recycling into the water column via the contaminated sediments (*39*). Contaminants that are dissolved or associated with colloidal particles can exchange across sediment-water interface by diffusive or advective processes (*40, 41*). Subsequently, the sediments as a depositional reservoir for historically discharged contaminants could have become an important source of contamination and continued to recycle deposited contaminants to the overlying water column (*42*). The presence of bioavailable p, p'-DDT and its metabolites presumably resulted from historically DDT-deposited sediments. The findings suggest that historically contaminated sediments rather than external non-point sources have become the dominant source of DDT contamination in Portland Harbor.

Dominance of bioavailable p, p'-DDD in surface water. The dominance of bioavailable p, p'-DDD over bioavailable p, p'-DDE was observed. Figure 2.2 reveals p, p'-DDD is the most abundant bioavailable DDT derivatives followed by p, p'-DDE, and p, p'-DDT. The average percentage contributions of p, p'-DDD, p, p'-DDE, and p, p'-DDT to ΣDDTs in the samples from eleven sampling sites in four years were 53 ± 14%, 35 ± 11%, and 13 ± 6%, respectively. In general, the principal insecticidal ingredients of technical DDT contained p,p'-DDT 72%, o, p'-DDT 20%, p,p'-DDD 3%, o, o'-DDT 0.5% and other 4.5% (*43*). Both p, p'-DDD and p, p'-DDE existed as by-products in technical DDT and have been formed by environmental degradation of DDT. The high contribution of p, p'-DDD and p, p'-DDE in the bioavailable fraction suggested a large proportion of p, p'-DDT has been transformed to p, p'-DDD and p, p'-DDE. It is noteworthy that p, p'-DDD was also marketed as an insecticide in its own right, as well as, being a reductive metabolite of p, p'-DDT (*43*). In addition to being a reductive metabolite of p, p'-DDT, a large proportion of p, p'-DDD

could therefore indicate a direct input of p, p'-DDD in this area. The results indicated p, p'-DDD was the most bioavailable p, p'-homolog in surface waters of the lower Willamette River

The large bioavailable concentrations of ΣDDTs coincided with large DDD/ DDE ratios (Figure 2.3). The ratio of DDD/DDE was highly variable between sites and sampling periods. The DDD/DDE ratio at River Mile 7 West was highly variable, 6.6 ± 2.1 in summer, and 2.8 ± 1.3 in fall. The large proportion of bioavailable p, p'-DDD and the large ratio of bioavailable DDD/DDE in surface waters could be governed by water solubility and the partition coefficients (log K_{ow} and log K_{oc}). Water solubility of p, p'-DDT, p, p'-DDD and p,p'-DDE are 0.0055 mg/L, 0.02 mg/L, and 0.1 mg/L at 20-25 °C (*44*) . However, the bioavailable p, p'-DDT, p, p'-DDD, and p, p'-DDE concentrations in this study were measured at the level much below the water solubility and therefore water solubility is unlikely to be the cause of the observation. Log K_{ow} ; 5.7 for p,p'-DDT, 6.1 for p, p'-DDD, 6.0 for p, p'-DDE are similar, as well the sorption coefficients (log K_{oc}); 6.3 for p, p'-DDT, 5.0 for p, p'-DDD, 4.7 for p, p'-DDE at 20-25 °C (*32, 44*) are not likely to account for the observation seen. It is unlikely that the large proportion of bioavailable p, p'-DDD and large ratios of bioavailable DDD/DDE are the results of differences in solubility or partitioning, suggesting the large bioavailable p, p'-DDD concentrations may be influenced by other factors.

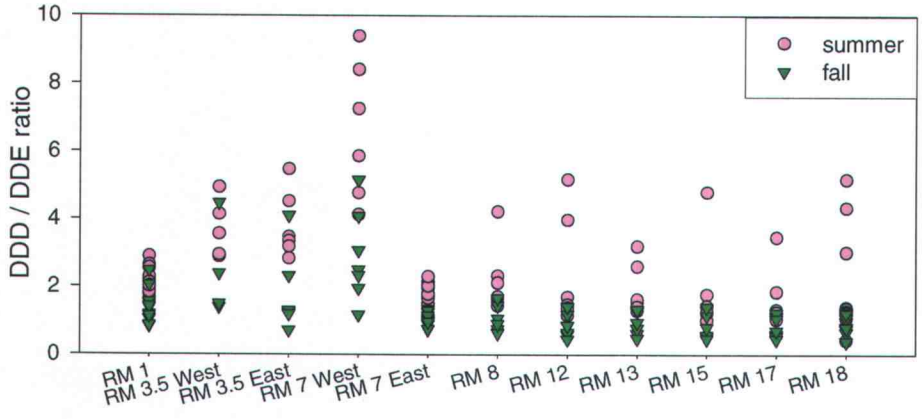

Figure 2.3 Distributions of bioavailable p, p'-DDD: p, p'-DDE ratios in surface waters at the Willamette River, Oregon during the periods of low flow in summer, and high flow in fall from 2001 to 2004 (n=186).

The large ratio of DDD/DDE was previously observed in the Portland Harbor sediments from River Mile 7-8 West (*15*). Zeng and Tran (*42*) showed evidence for a strong tendency for organochlorines including DDTs, to move from sediment to overlying water. The similarity of DDT profiles in the sediment (*15*) and the water bioavailable fraction supports the possibility of the movement of bioavailable DDT and its metabolites to the water column from historically contaminated sediments. The large ratio of DDD/DDE could also indicate the potential for reductive dechlorination of DDTs to DDDs in sediments under flooded, anaerobic conditions (*45, 46*). Under anaerobic conditions in sediments, DDT is mainly metabolized to DDD by reductive dechlorination either by microbial degradation or by chemical reaction while under aerobic condition, DDT is metabolized to DDE by dehydrochlorination (*47-49*). It is possible that the lower Willamette River at Portland Harbor which is a deep and slow moving river (*15*) has a potential condition for anaerobic reductive dechlorination of DDT to DDD rather than to DDE. In general, p, p'-DDE tends to resist further degradation as compared to p, p'-DDD (*48, 49*). However, Quensen et al. (*50*) found that in anaerobic marine sediments, DDE was dechlorinated to DDMU (1-chloro-2,2-bis-(4'-chlorophenyl)-ethylene) three orders of magnitude faster than the transformation of DDD to DDMU. In addition to direct input of DDD, anaerobic reductive dechlorination of DDT to DDD and possible persistence to further degradation of DDD under anaerobic condition in sediments may all be responsible for the enrichment of bioavailable p,p'-DDD in Portland Harbor. However, further investigations of dechlorination conditions in the harbor sediment are warranted.

Evidence of seasonality in bioavailable ΣDDT concentrations. Seasonal variations of bioavailable ΣDDTs at the lower Willamette River at Portland Harbor were observed. Seasonal variation of bioavailable ΣDDTs is site-specific. Seasonal variation of bioavailable Σ DDT concentrations was only statistically different at River Mile 7 West ($p = 0.002$, t-test) (Figure 2.2). A 2-fold decrease of bioavailable ΣDDTs during periods of high flow in fall was measured at River Mile 7 West. The decrease in fall is due to a decrease of bioavailable p, p'-DDD. In contrast, bioavailable p, p'-DDT and p, p'-DDE did not decrease in fall. By contrast to River Mile 7 West, bioavailable ΣDDT concentrations at the other sites remained unchanged between summer and fall.

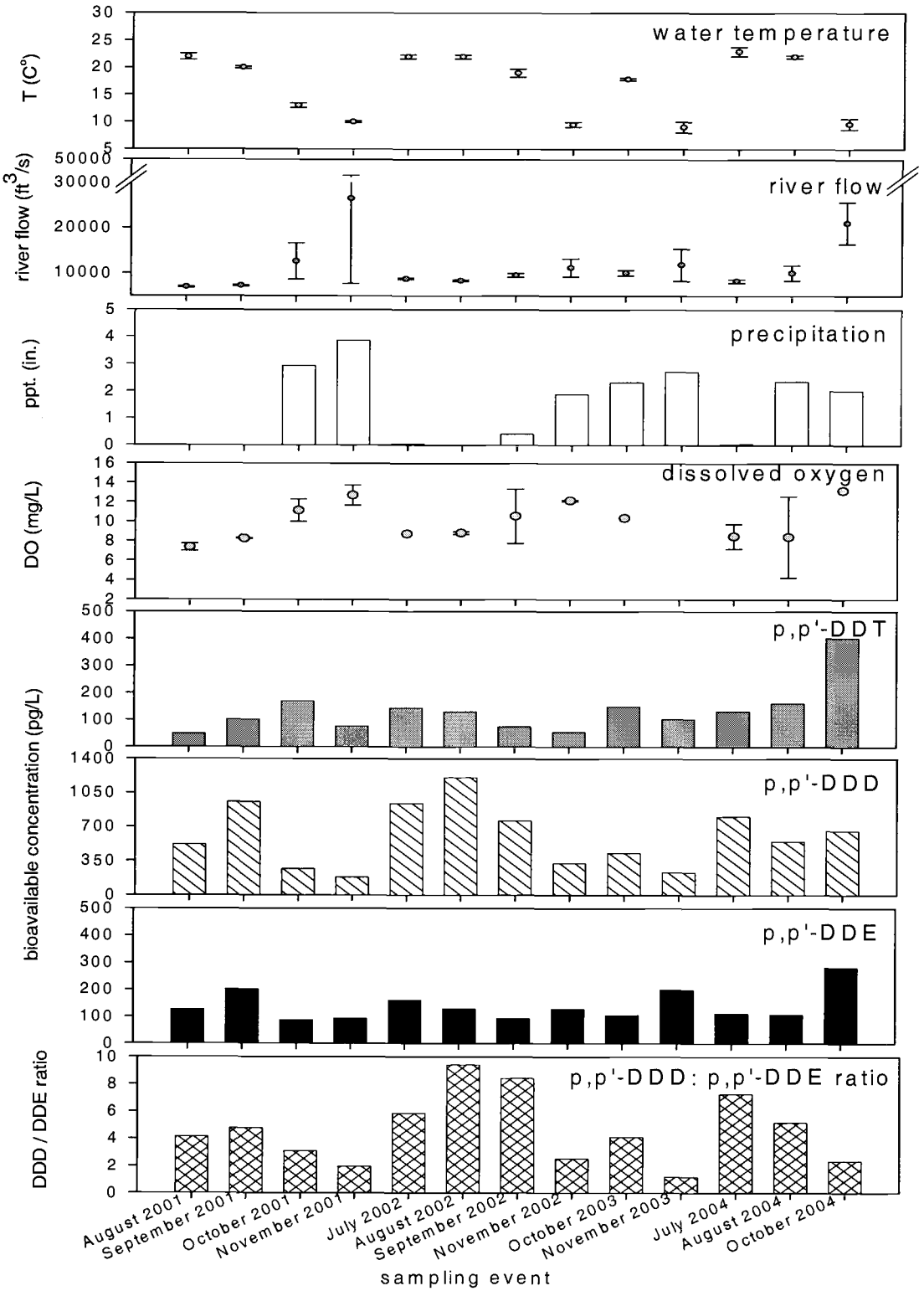

Figure 2.4 Estimated concentrations of bioavailable p, p'-DDT and its metabolites in surface water at River Mile 7 West of the lower Willamette River at Portland Harbor, Oregon in relation to average water temperature, average river flow, total precipitation, total organic carbon (TOC, black circle), and dissolved organic carbon (DOC, white circle) concentrations.

At River Mile 7 West, bioavailable ΣDDT concentrations were significantly lower in fall ($p = 0.002$, t-test), excluding October 2004 (Figure 2.4). Bioavailable p, p'-DDT, p, p'-DDD, and p, p'-DDE concentrations in October 2004 were unusually high compared to the concentrations measured in fall 2001 to 2003 and were treated as an outlier in this case. In general, a significant decrease of bioavailable p, p'-DDD ($p = 0.001$, t-test) in fall appeared to be a major driver of seasonality in bioavailable ΣDDT concentrations. Bioavailable p, p'-DDT and p, p'-DDE were statistically unchanged between seasons ($p = 0.58$ for p, p'-DDT and $p = 0.50$ for p, p'-DDE, t-test). The bioavailable p, p'-DDD concentration was positively correlated with average water temperature ($r^2 = 0.62$, $p = 0.003$), and negatively correlated with average river flow ($r^2 = 0.39$, $p = 0.03$) and total precipitation ($r^2 = 0.71$, $p < 0.001$) (Figure 2.4). As dissolve oxygen concentration is a function of water temperature, bioavailable p, p'-DDD was also negatively correlated with dissolved oxygen concentration ($r^2 = 0.49$, $p = 0.016$). Bioavailable p, p'-DDD concentration was not statistically correlated to total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations (Figure 2.4). The results suggest seasonal change in bioavailable p, p'-DDD concentrations is potentially related to changes in temperature, river flow, and/or precipitation.

The average amount or load of bioavailable DDTs transported in surface water at the lower Willamette River (River Mile 1-18) during the 4- year study in summer ranged from 1.1 g/d to 22 g/d for bioavailable ΣDDTs; 0.11 g/d to 2.1 g/d for bioavailable p, p'-DDT; 0.61 g/d to 18 g/d for bioavailable p,p'-DDD; and 0.36 g/d to 2.7 g/d for bioavailable p,p'-DDE. The average loads during fall were 2.6 g/d to 27 g/d for bioavailable Σ DDTs; 0.26 g/d to 6.9 g/d for bioavailable p, p'-DDT; 0.86 g/d to 15 g/d for bioavailable p, p'-DDD; and 1.3 g/d to 6 .0 g/d for bioavailable p, p'-DDE. The highest loads of bioavailable DDTs always observed at River Mile 7 West during summer and fall. In general, the loads of bioavailable p, p'-DDT and its metabolites were not significantly different between seasons ($p > 0.05$, t-test).

Daily average river flow and water temperature were used to evaluate the seasonality of bioavailable p, p'-DDD concentrations and ratios of bioavailable DDD/DDE (Figure 2.4). An increase of bioavailable p, p'-DDD concentrations occurred during the low flow in summer with a maximum observed during extended dry periods. Simply, one might be

tempted to think dilution could account for the decrease in bioavailable p,p'-DDD concentrations during fall sampling events when the river flow is large. However, if dilution was the cause, dilution should decrease all analytes similarly; this was not what we found. Both bioavailable p, p'-DDT and p, p'-DDE were found to have the same or higher concentrations in fall as compared to summer. Also, the DDD/DDE ratio should not change if dilution is a major cause. It appeared that physical-chemical processes governed by water temperature and river flow variations alone could not explain the observed seasonal variation in bioavailable p, p'-DDD. We hypothesize that the seasonal distribution of bioavailable p,p'-DDD is governed by a combination of physical and biological factors that vary seasonally as a function of temperature, flow velocities, and aquatic organism activities.

The occurrence of elevated bioavailable p,p'-DDD concentrations and bioavailable DDD/DDE ratios in mid July to mid September coincided with elevated temperature. Increase in water temperature could decrease the organic matter-water partition coefficient (K_{om}) of contaminants (*51*). Schwarzenbach et al. (*51*) estimated increasing temperature by 10 °C could result in an estimated 30% decreased absorption coefficient for pyrene. The effect of increasing temperature by approximately 10 °C in summer could result in an increased dissolved fraction from the sediment-water exchange for ΣDDTs. A 30% increase in bioavailable ΣDDTs release would result in elevated concentrations of ΣDDTs in surface water. However, it appeared that increase of ΣDDTs release from the contaminated sediment due to decreasing temperature-dependent absorption coefficients alone might not readily explain the significant increase in DDD/DDE ratios observed in summer. This gap suggests another influencing factor(s) that could contribute to the elevated concentrations of bioavailable p, p'-DDD in summer.

Increased water temperature would also be expected to increase growth and activity of benthic organism, microorganisms, eutrophication, and bitoturbation. Bioturbation by burrowing organisms at the surface sediment can reintroduce sediment-borne chemicals to the water column (*52*). Some aquatic plants can uptake and transform p, p'-DDT primarily to p, p'-DDD (*53*). Increasing aquatic plant growth could lead to oxygen depletion as microorganisms break down the dead tissues. The top layer of the sediment may be aerobic or anaerobic depending on the condition of the water column above; however the sediments

quickly become anaerobic with increasing sediment depth (*52*). There was also declining redox condition ($p = 0.054$, *t*-test) as water temperature and dissolved oxygen concentrations changed in summer (Table 2.1), a contributing factor to enhance anaerobic condition in the sediments. Anaerobic reductive condition is the dominant condition for reductive dechlorination of p, p'-DDT to p, p'-DDD by both microbial degradation and chemical reaction (*47-49*). Slow moving surface waters during summer would enhance anaerobic reductive degradation to p, p'-DDD as Kale et al.(*45*) found p, p'-DDD was a major metabolite of p, p'-DDT detected in sediment and overlying water under flooded condition. Evaluated together, increasing formation and release of p, p'-DDD as result of increased temperature, decreased dissolved oxygen concentration, and the condition favoring reductive conditions in sediment during low flow in summer would contribute to an increase of bioavailable p,p'-DDD and high ratio of DDD/DDE in surface water. As river flow and precipitation increased in early fall, the formation and release of p, p'-DDD could be affected by dilution process as suggested by relatively no change in DDD/DDE ratio. Decreasing ratio of DDD/DDE in fall suggested seasonal influence on p, p'-DDD sources. As water temperature decreased and river flow increased in fall, the condition suitable for reductive dechlorination may have been altered resulting in decreased bioavailable p, p'-DDD concentrations and change in bioavailable DDD/DDE ratios.

The above discussion contains speculation of the processes that highlights the lack of understanding on how seasonal river conditions interact to yield the observed seasonal variation in bioavailable p, p'-DDD and its relationship to bioavailable p, p'-DDE. Further research is needed. Understanding the interaction of seasonal physico-chemical and biological factors will afford confidence in using this knowledge to predict the long-term seasonal effects of ΣDDTs on aquatic community and increased confidence in extrapolating information to other contaminated sites.

Potential risk of seasonal bioavailable ΣDDTs on aquatic organisms. The present study measured the bioavailable concentrations of ΣDDTs in surface water which allowed a direct assessment of the water quality in the Portland Harbor. Aquatic organisms can take up large amount of ΣDDTs over time from surrounding water where ΣDDTs are present at very

low concentrations (*54*). It has been addressed that ΣDDTs taken into aquatic organisms has come from surrounding water while a large proportion of Σ DDTs in their body has come from the food (*54*). To assess the potential impacts of seasonal distribution of ΣDDTs on aquatic organisms at the lower Willamette River, water concentration estimates were compared to the national recommended water quality criteria (*22*) and the Oregon water quality criteria (*23*) (Figure 2.6). Exceedances of the fresh water aquatic life criteria for ΣDDTs (1,000 pg/L for both national and Oregon criteria) were seasonally influenced and limited to the samples from RM 7 West. The bioavailable concentrations of bioavailable ΣDDTs always exceeded the national and the Oregon criteria during low flow in summer. During summer, the aquatic organisms at RM 7 West could have been exposed to a greater amount of ΣDDTs, particularly p, p'-DDD, due not only to seasonally increased exposure concentrations but also to increased uptake rate. As aquatic organisms are ectothemic, the rate of metabolism undergoes an approximately 2-fold increase with every 10 °C rise in water temperature (*55*). Cairns et al (*55*) suggested increased temperature could change respiratory rate and membrane permeability and thereby leaded to more rapid uptake and hastened the accumulation of a toxic dose. The effect of increasing temperature approximately by 10 °C in summer could result in increased ΣDDT bioconcentration from surrounding water. Water concentrations determined in this study are the bioavailable fraction which has been known as the uptaken form by aquatic organisms. Therefore, exceedance of ΣDDT fresh water aquatic life criteria, as well as, the potential to increase their ability to bioconcentrate ΣDDTs from surrounding water during warm weather can pose a potential risk for ΣDDT chronic toxicity to aquatic communities at River Mile 7 West.

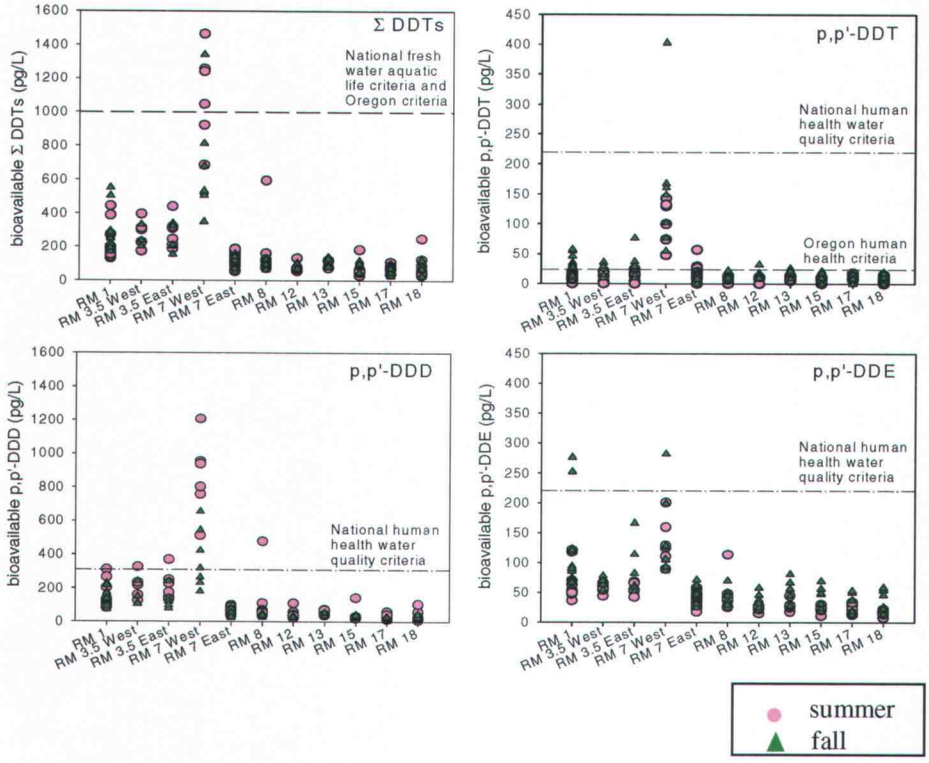

Figure 2.6 Spatial and seasonal distribution of bioavailable p, p'-DDT and its metabolite water concentration estimates compared to the national recommendation water criteria (*22*) and Oregon criteria (*23*) to protect aquatic organism from chronic exposure to ΣDDTs and to protect local fish consumers who consume fish and shellfish from the lower Willamette River at Portland Harbor, Oregon.

Bioavailable concentrations of p, p'-DDT, p, p'-DDD, and p, p'-DDE were also compared to the national and Oregon human health water quality criteria to estimate life-time cancer risk for local fish consumers based on a 10^{-6} increased lifetime cancer risk (Figure 2.6). Exceedances of the human health water quality criteria for p, p'-DDT and its metabolites were mostly observed at RM 7 West. Although no samples exceeded the national criteria for p, p'-DDT (220 pg/L), all samples from RM 7 West exceeded the Oregon criteria (24 pg/L). Exceedance of the national human health water quality criteria for p, p'-DDD (310 pg/L) was seasonally affected. Bioavailable concentrations of p, p'-DDD tended to exceed the criteria during low flow in summer sampling events. Only a few samples exceeded the criteria for p, p'-DDE (220 pg/L). Although, the human health water quality criteria is not the direct approach for human health risk assessment for fish consumers but it is useful as a screening approach for indicating potential areas of concern. According to the results in our

study, the potential adverse effects of ΣDDTs for local fish consumers were realized locally at River Mile 7 West. This finding agreed with the bioaccumulation profiles of ΣDDTs in fish and human health risk estimates for ΣDDTs (2×10^{-6} to 8×10^{-5}) from consuming fish in this area in our previous study (*17, 18*). The excellent agreement between the present study and our previous fish study confirmed passive sampling devices can be used as a surrogate to study bioavailable distribution of organic contaminants in contaminated water.

Distribution of bioavailable polychlorinated biphenyls (PCBs). In contrast to the generally point source nature of DDT contamination, there were several former PCB using facilities densely located on both sides of the river from RM 2-10.5 (*56*). These facilities have been identified as potential sources of PCB contamination in the harbor. Elevated concentrations of PCBs were detected in sediments and subsurface sediments from RM 3.5 to 9.5 (*15*). Dissolved PCBs or colloidal particle-associated PCBs in the sediment can exchange across sediment-water interface by diffusive or advective processes, subsequently, recycling historically deposited PCBs to the water column (*40-42*). In addition, the current major sources of PCBs to urban rivers include direct urban runoff, urban community wastewater discharge, sewage treatment works, and combined sewer flow discharges (*57-59*). Considering the influence of multiple PCB local sources and upland land uses, the sampling sites were then grouped into three locations; RM 1-8 (industrial area including most of Portland Harbor superfund site), RM 12-13 (urban area) and RM 15-18 (residential area). The spatial pattern of bioavailable PCB concentrations in water coincided with the historically spatial PCB sediment distribution. A spatial pattern of bioavailable ΣPCB concentrations was observed in summer and fall (Figure 2.7). Bioavailable concentrations of ΣPCBs ranged from below detection limits (0.69-54 pg/L depending on congeners) to 410 pg/L at the eleven sampling sites from River Mile 1-18 during the four-year study with an average concentration of 54 ± 55 pg/L. In general, higher bioavailable ΣPCB concentrations occurred in the industrial and urban areas from River Mile 1-13. Elevated concentrations of bioavailable Σ PCBs usually reached a maximum at RM 3.5 East, the lower superfund site. Average bioavailable concentrations of ΣPCBs during low flow in summer ranged from 47 - 74 pg/L at the industrial area, from 26-33 pg/L at the urban area and from 12-15 pg/L at the residential area. Average bioavailable concentrations of ΣPCBs during high flow in fall

ranged from 37-170 pg/L at the industrial area, from 36-69 pg/L at the urban area and from 23-42 pg/L at the residential area.

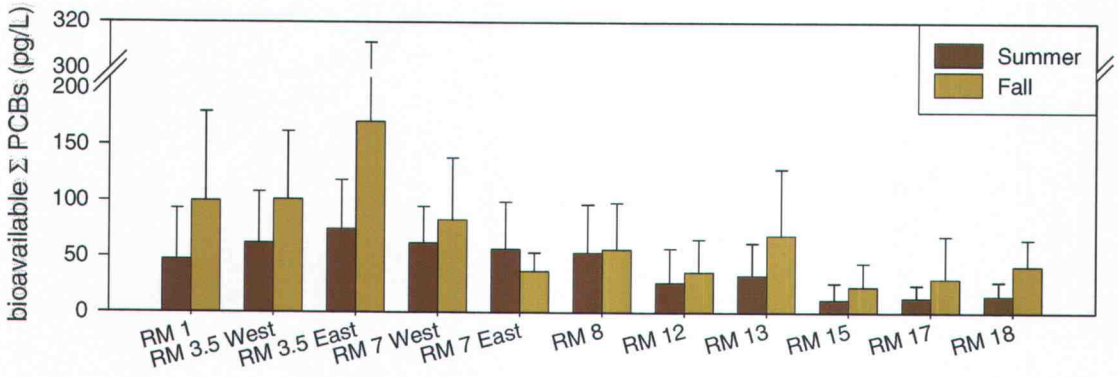

Figure 2.7 Averaged concentration estimates of bioavailable ΣPCB (pg/L) in water at the Willamette River, Oregon according to river season (low flow, low precipitation in summer and high flow, high precipitation in fall) in 2001 to 2004. The error bars denote 1 SD.

The bioavailable concentrations of ΣPCBs in the lower Willamette River were generally comparable with or lower than the dissolved concentrations determined in other large water bodies in US including San Diego Bay (11 – 330 pg/L based on 27 PCB congeners) (*60*), Lake Superior (90 ±10 pg/L based on 25 PCB congeners) (*61*), Lake Michigan (340 -1700 pg/L based on 85 PCB congeners) (*62*), the Susquehanna River, Maryland (500 -5300 pg/L based on 85 individual congeners) (*10*), and much lower than those in some river systems around the world such as Minjiang River, China (200,000 – 2,500,000 pg/L based on 21 PCB congeners) (*36*). It is noted that comparison between PCB contamination at the lower Willamette River and the other rivers did not account for the variations from sampling technique used, the techniques used to extract the operationally-defined dissolved phase and numbers of congeners analyzed. Other techniques probably overestimate the bioavailable concentrations.

Seasonal changes in bioavailable ΣPCBs distributions. Bioavailable concentrations of ΣPCBs showed a discernible seasonal pattern at almost every sampling site (Figure 2.7). A two-fold increase in bioavailable ΣPCB concentrations was observed during the high flow in fall, with the residential site (RM 15-18) statistically significant ($p = 0.003$, t-test). The average daily amounts or loads of bioavailable ΣPCBs transported in surface water at the lower Willamette River also showed significant seasonal differences. Average of bioavailable ΣPCB daily loads in surface waters were significantly higher in fall; industrial area ($p = 0.002$, t-test), urban area ($p = 0.043$, t-test), and residential area ($p = 0.001$, t-test). The average of bioavailable ΣPCB daily loads at the industrial area (River Mile 1-8) during high flow in fall was 3.4 g/d, compared to 1.2 g/d during low flow in summer. At the urban area (River Mile 12-13) and residential area (River Mile 15-18), the average of bioavailable ΣPCB daily loads during high flow in fall was 2.56 g/d and 1.46 g/d, compared to 0.62 g/d and 0.28 g/d during low flow in summer. In spite of the dilution effect, bioavailable water PCB concentrations were larger during high flow in fall than during low flow in summer.

Bioavailable PCB composition in surface water. The average percent congener distributions of bioavailable PCBs are shown in Figure 2.8. PCB 153 and PCB 138 (hexa-CB homolog) were the most frequently detected bioavailable congeners with 85% and 73% frequency, respectively. PCB 49 (tetra-CB) was the most abundant bioavailable congeners followed by PCB 52 (tetra-CB) and PCB 153 (hexa-CB) as the second most abundant bioavailable congeners detected. PCB 166 and two of the co-planar, dioxin-like congeners, PCB 77 and PCB 126 were below the detection limits in all samples.

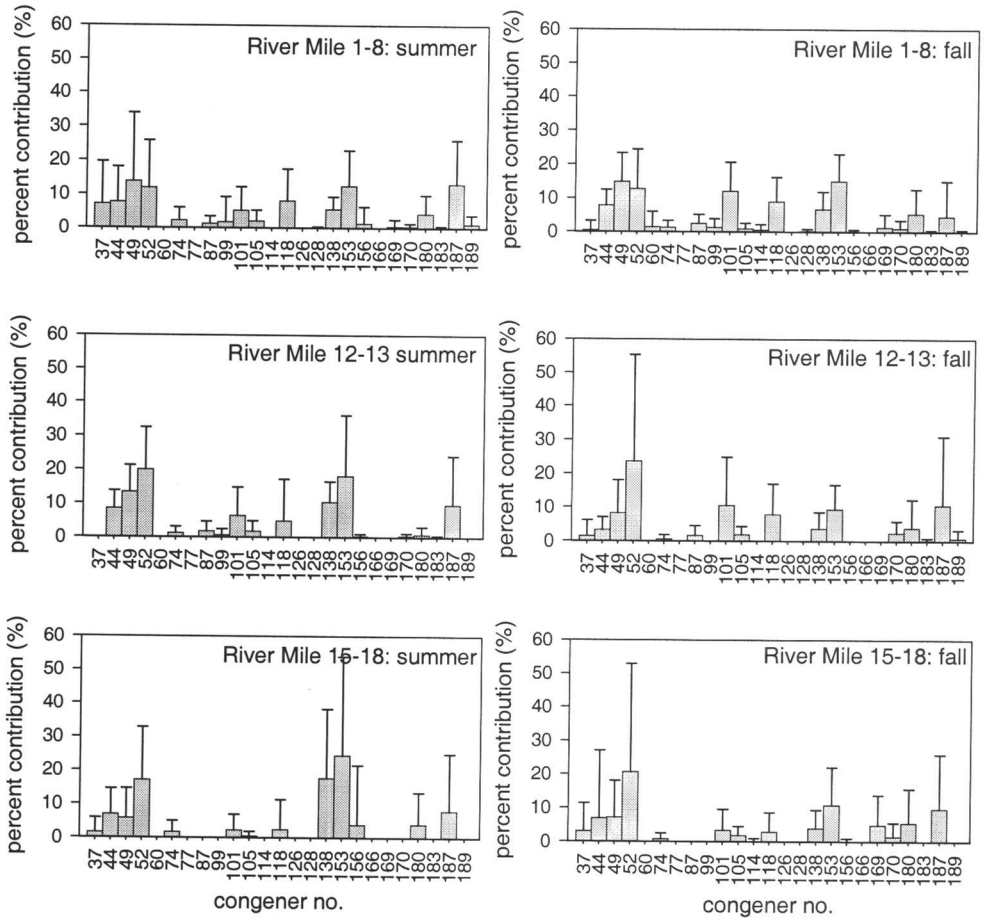

Figure 2.8 Average percent contributions of PCB congeners in surface water at the lower Willamette River during the periods of low flow in summer and high flow in fall in 2001 to 2004. The error bars denoted 1 SD.

Although bioavailable ΣPCB concentrations and loads were found to have strong spatial and seasonal variation, the PCB congener profiles did not. The congener profiles for River Mile 1-8 and River Mile 12-13 were fairly uniform and no discernable seasonal patterns were observed, Figure 2.8. The similarity of PCB congener profiles for the industrial area at the superfund site (River Mile 1-8) and for the urban area upstream (River Mile 12-13) suggests the local sources of PCBs at the superfund site are important enough to significantly increase the concentrations of bioavailable PCBs in surface water but not to substantially change composition relative to the surface water inputs from upstream.

The average percent congener distributions for River Mile 15-18 relative to the average of the other sampling sites showed the bioavailable PCBs to be comprised of a slightly different composition, with the distribution maximum shifted toward the hexa homolog (PCB 138 and PCB 153) as compared to the other sampling sites, which favored the tetra homologs (PCB 44, PCB 49 and PCB 52), Figure 2.8 and 2.9. However, the average percent congener and homolog distributions at River Mile 15-18 became similar to those at the other sampling sites in the periods of high flow in fall.

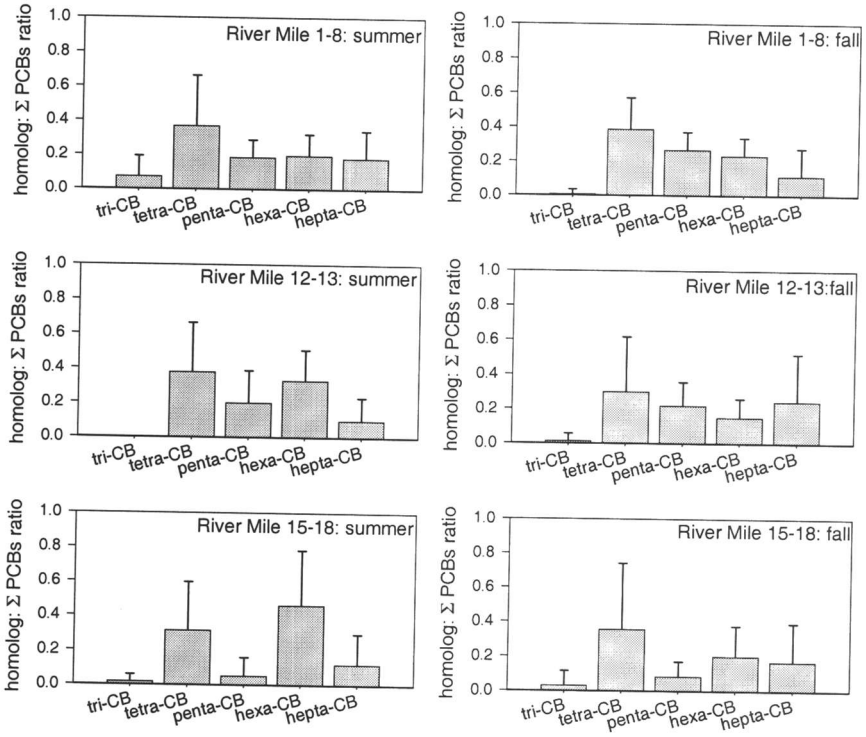

Figure 2.9 Average ratios of PCB homolog to bioavailable ΣPCBs (sum of 25 individual PCB congeners) in surface water at the lower Willamette River during the periods of low flow in summer and high flow in fall in 2001 to 2004. The error bars denoted 1 SD. See detail of PCB homologs in text.

The present study suggests the sources of bioavailable PCBs in surface waters at the lower Willamette were presumably dependent on seasons. Storm flow was especially important. Both concentrations and daily loads of bioavailable PCBs increased during high

precipitation and high river flow, especially during episodic rainstorms in November of 2001 and October of 2004. The importance of high river flow enhancing PCB transport in surface waters has been previously described (*10*). Foster et al. (*10*) demonstrated PCB sorption coefficients (K_ds) were decreased during storm flows and subsequently enhanced the dissolved, or the "bioavailable" PCB concentrations in the surface waters. They indicated the lower K_ds of PCBs during storm flows may be attributed to an increase in DOC, a change in particle organic matter content, or shifts in PCB congener profiles to a greater extent of the less chlorinated congeners. In the present study, DOC and TOC concentrations at the lower Willamette River did not change seasonally over the 4 year-study period (Table 2). DOC and TOC concentrations were not correlated with the bioavailable PCB concentrations. There was no evidence of seasonal or river flow-related change in PCB congener or PCB homolog profiles observed at River Mile 1-8 where an average 50% increase in bioavailable Σ PCB concentrations was observed. In contrast, a shift in PCB congener and homolog compositions toward the less chlorinated congeners was observed at River Mile 12-13 and River Mile 15-18. High river velocities during storms can promote the transport of a relatively larger fraction of course particles in particulate phase which have lower organic carbon contents (i.e., changing organic matter content) and reduced PCB sorption(*63*). The results suggests storm events (i.e., high river flow, high precipitation) may have disturbed the interaction between PCBs and their associated organic matters resulting in remobilization of bioavailable fraction from sediments or particulate matters.

Additional inputs during high flow in fall such as precipitation and storm water runoff may have also contributed to elevated bioavailable ΣPCB concentrations and PCB composition changes during high flow in falls. Atmospheric transport including precipitation is an important pathway for the transfer of PCBs to surface water as indicated in several studies (*12, 64, 65*). Bremle and Larsson (*12*) reported precipitation was a dominant source of PCBs in a Swedish river during high flow. The PCB congener and homolog profile in surface water samples from the lower Willamette River were similar to that observed in rain samples in other studies, which were dominated by tetra-chlorinated congeners, including PCB 52 (*64, 65*). The similarity of PCB homolog patterns in our study and those in rain samples by others may suggest precipitation inputs as an additional potential source of PCBs into the river system during high precipitation and further investigation is needed.

Urban storm waters and urban community wastewater discharges are another source of PCB contamination in the surface waters (*57, 59*). Rossi et al (*57*) reported urban storm water was a major contribution to PCBs in Swiss urban water systems. In the New York/New Jersey Harbor Estuary, USA, PCB concentrations in the influent were elevated during storms(*59*). Approximately, 3% of the annual PCB contribution from the wastewater treatment plants were diverted and bypassed the wastewater treatment plants and discharged through combined sewage overflow due to precipitation events(*59*). In the lower Willamette River at Portland, combined sewer overflows to the Willamette River occur frequently (i.e., 100 days or 50 events overflow in 2004), especially during periods of high precipitation which usually start in mid October(*66*). The increase in bioavailable PCB concentrations and loads which was coincident with high precipitation and sewer overflows (Table 2.1) may suggest a significant contribution of PCBs from urban storm waters and urban community wastewater discharges to the surface waters. The hypothesis that combined sewer overflows and precipitation could be a major source of PCBs during high precipitation and high river flow in the lower Willamette River warrants further investigation such as PCB fingerprints to fully elucidate their contribution to PCB contamination. Further studies will be necessary to estimate the impact of the internal sources (i.e., contaminated sediments) and other sources (i.e., precipitation, runoffs, wastewater discharges) of PCBs and how much they contribute to the PCB problem in the system in order to reduce the PCB load and risk to the aquatic organism community.

PCBs-associated risks to aquatic community and human health. None of samples exceeded the national and the Oregon fresh water aquatic life criteria for ΣPCBs (14000 pg/L). In contrast, exceedances of the national (64 pg/L) and the Oregon human health water quality criteria (79 pg/L) for fish consumers were often observed at the sampling sites within the superfund site (River Mile 1-8) (see Figure 2.10) but no discernable seasonal pattern was observed. Year-round exceeding the human health water quality criteria suggests consuming fish and shellfish from this area may pose adverse health effect risk to recreational or subsistence fishers based on a 10^{-6} increased lifetime cancer risk for ΣPCBs. This suggestion is consistent with risk estimates (9×10^{-6} to 5×10^{-3}) for ΣPCBs from consuming fish in this area in our previous human health risk assessment study (*17*). The extent of exceedance would have been greater if more PCB congeners were included for the measurement.

Nevertheless, this exceedance indicates that PCB contamination remains a significant problem at the Portland Harbor superfund site.

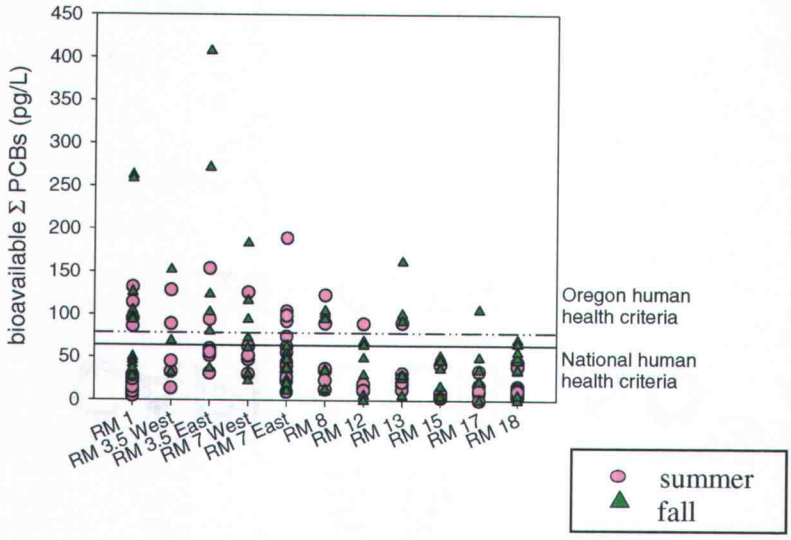

Figure 2.10 Spatial and seasonal distribution of bioavailable ΣPCB water concentration estimates (sum of 25 individual PCB congeners) compared to the national recommendation water criteria (*22*) and Oregon criteria (*23*) to protect local fish consumers who consume fish and shellfish from the lower Willamette River at Portland Harbor, Oregon.

Seasonal changes in bioavailable dieldrin distribution. Bioavailable dieldrin was detected at all sampling sites. Over the 4-year study period, the ranges of bioavailable dieldrin concentrations in the mainstreams of River Mile 1-18 of the lower Willamette River were from 10 to140 pg/L. No discernable spatial pattern was observed for dieldrin. However, river condition changes between low flow in summer and high flow in fall appeared to influence the distribution of bioavailable dieldrin in the surface water. An increase in bioavailable dieldrin concentrations was found at most sampling sites during periods of high river flow, high precipitation in fall ($p \leq 0.046$, *t*-test), Figure 2.11. Bioavailable dieldrin daily loads were also found to be higher in fall. The average daily loads of bioavailable dieldrin ranged from to 1.5 to 3.8 g/d during high flow, compared to 0.63 to 1.3 g/d during low flow. The increase in both bioavailable concentrations and loads of dieldrin were

coincident with high precipitation and high river flow. It is noted that the increases were higher at the sampling sites upstream, particularly at RM 18 which was downstream of Johnson Creek, an agricultural/urban creek. The seasonal pattern of bioavailable dieldrin distribution illustrates the transport behavior for non-point source contaminants which are widely dispersed and have no intentional seasonal application cycle such as with the current use pesticides. Dieldrin was widely used in the Willamette basin before it was banned and Johnson Creek has been found to be one of the most dieldrin contaminated areas in the watershed (*67*). Elevated concentrations of bioavailable dieldrin during high flow and storm events in fall suggests bioavailable dieldrin transport from contaminated site upstream including erosional inputs to the river during precipitation runoff from land sources or riverbed sediment (*68*).

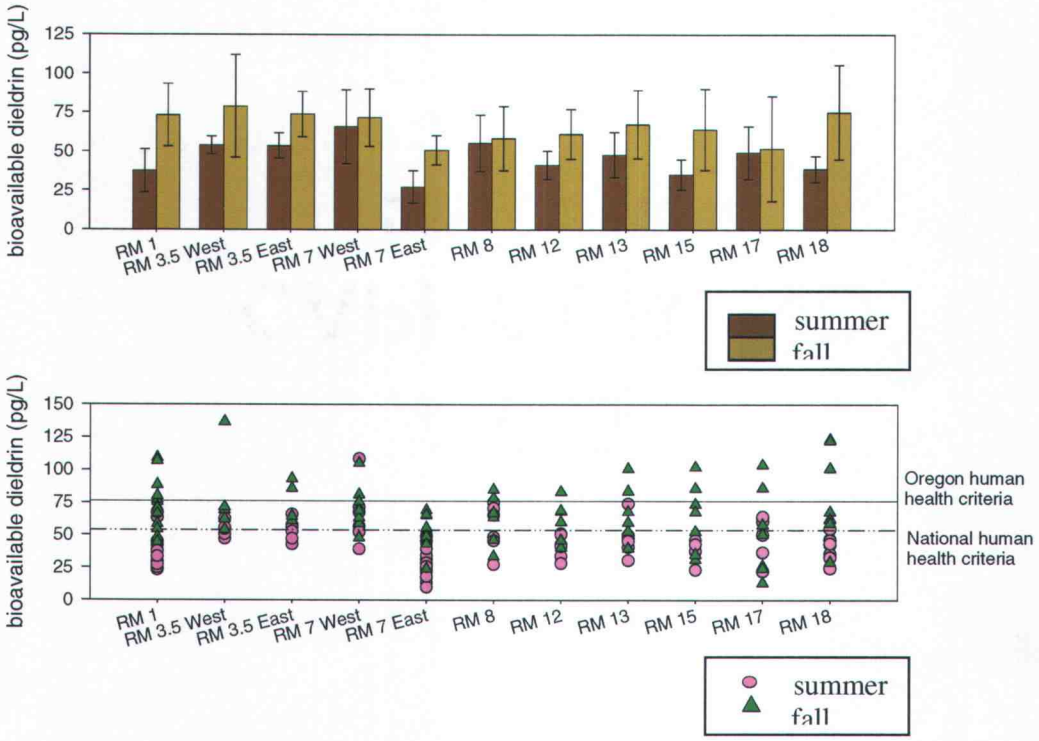

Figure 2.11 Seasonal distribution of bioavailable dieldrin in surface water at the lower Willamette River at Portland Harbor, Oregon during the periods of low flow in summer and high flow in fall of 2001-2004. The top panel shows average concentration estimates of bioavailable dieldrin while the bottom panel shows their distribution compared to the national recommendation water criteria (*22*) and Oregon criteria (*23*) to protect local fish consumers who consume fish and shellfish from this area.

Bioavailable dieldrin concentrations were compared to the national recommended water quality criteria (*22*) and the Oregon water quality criteria (*23*). None of samples exceeded the national (56000 pg/L) and the Oregon fresh water aquatic life criteria (1900 pg/L) for dieldrin. Exceedances of the national and the Oregon human health water quality criteria for dieldrin were frequently observed in fall, particularly at the sampling sites upstream. This finding is consistent with risk estimates (2×10^{-6} to 3×10^{-5}) for dieldrin from consuming fish in this area in our previous human health risk assessment study (*17*).

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CHAPTER 3

FIELD TRIAL OF TRIOLEIN-FREE LOW-DENSITY POLYETHYLENE MEMBRANE AS AN IN SITU PASSIVE WATER SAMPLER

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Abstract

Responding to a growing need for an inexpensive and simple time-integrative sampling device for bioavailable hydrophobic toxic contaminants in water, a triolein-free low density polyethylene membrane lay flat tubing (LFT) has been further developed. The LFT sampler is similar to semipermeable membrane device (SPMD) based on the diffusion of dissolved/bioavailable target hydrophobic compounds through the aqueous boundary layer and polyethylene membrane and the subsequent accumulation in the membrane and a neutral lipid reservoir (i.e., triolein), mimicking uptake by living organisms. Unfortunately, the interference stemming from the triolein impurities in SPMD has sometimes impeded the analyses and some target analytes are sacrificed in the removal processes of triolein impurities. Here, we demonstrate laboratory and field verification that the LFT without triolein proved reliable and had the same benefits as SPMD, but was simpler, inexpensive and lacked interference from the triolein impurities. A total of 370 LFTs and SPMDs were deployed as a paired study at thirteen sampling sites in three different river conditions, 21-day deployment, at the Portland Harbor superfund site, Oregon. LFT has shown the ability to detect polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine (OC) pesticides as efficiently as the SPMD. Its sampling efficiency suggested the neutral lipid reservoir (triolein) present in SPMD was not generally necessary. Also, biofouling was not a problem for its field application as compared to SPMD. This simpler device tended to accumulate compounds with high log K_{ow} faster than SPMD. LFT sampling rates were estimated and modeled for 33 target analytes, including PAHs, PCBs, and organochlorine pesticides. Approaching equilibrium appeared to be not a concern even at a superfund site. The shortest duration estimated for compounds with log $K_{ow} \geq 4.4$ to maintain in linear uptake phase during LFT deployment is 24 days at water temperature 22 °C. A method for spiking permeability/performance reference compounds as an indicator for the effects of environmental conditions on the device uptake rate was achieved by using a simple spiking method in a small volume of toluene. The successful determination of field derived data illustrates the effectiveness and reliability of LFT for environmental monitoring.

Introduction

A wide range of passive sampling devices (PSDs) has been widely used in recent decades for monitoring exposure and assessment of contaminants in water, air, and soils(*1-11*). Semipermeable membrane device (SPMD) (*1*), solid-phase microextraction (SPME)(*2*), diffusive gradients in thin-films (DGT) (*3*), and other sampling formats with thin polymer coatings (*4, 8*) are examples of passive sampling technique developed and used for environmental assessment. The generally accepted major advantage of passive sampling technique over the conventional approach is the ability to distinguish between freely dissolved and bound molecules and focuses on the availability and activity rather than the mere presence of chemicals (*12*). The freely dissolved fraction is environmentally relevant to chemical bioavailability, toxicity, mobility and degradation process(*13, 14*). Instead of measuring the total concentration in a medium, the passive sampling technique measures the concentration in a reference phase which can be brought into equilibrium with the medium(*12*). The availability of a chemical is measured based on the chemical potential which is logarithmically related to its fugacity and linearly related to its freely dissolved concentration in a particular medium (*12*).

The semipermeable membrane device (SPMD) introduced by Huckins et al.(*1*) is a passive sampling technique that has been demonstrated to linearly sequester a variety of nonpolar and moderately polar organic contaminants (*15, 16*). SPMD has rapidly gained wide acceptance and has become commonplace for integrative sampling of environmental contaminants in water, air, and even soil (*2, 5, 7*). The device consists of low density polyethylene lay flat tubing containing a thin film of a neutral lipid, $\geq$ 95% pure triolein (1,2,3-tri[cis-9-octadecenoyl]glycerol) (*15, 16*). It has been proposed that SPMD mimics key mechanisms of bioconcentration including diffusion through biomembranes and partitioning between organism lipid and the surrounding medium (*15, 16*). Although SPMD provides many attributes such as a chemical bioavailability and simplicity of sample deployment and sample extraction, analysis of SPMD has sometimes been severely hampered by interferences stemming from polyethylene oligomers and triolein impurities (*17, 18*). Size exclusion chromatography can remove polyethylene oligomers and most of oleic acid and methyl oleate (*18*). However small amounts of oleic acid and methyl oleate remaining after size exclusion

chromatography are analytically problematic (*18*). Additionally, oleic acid in SPMD dialysates could contribute to false positive toxicity using Microtox assays (*19*). Petty et al (*15*) suggested a potassium silicate column can effectively removed oleic acid, unfortunately it can also remove target analytes (*18*). Gustavson et al (*17*) introduced a normal phase application of a disposable column containing a dual-zone restricted-access sorbent to separate methyl oleate from polyaromatic hydrocarbons (PAHs); however this method failed to separate methyl oleate from moderately polar to polar analytes(*18*). Lebo et al. (*18*) demonstrated purification procedure for triolein destined for use in SPMD could greatly reduce interferences caused by impurities in the triolein. Nonetheless the implementation of these efforts to reduce interferences stemming from the triolein impurities would be expensive and laborious and some contaminants of interest are often sacrificed.

In addition to triolein, the polyethylene membrane itself contained a significant portion of the total amount of dissolved target analytes accumulated by an SPMD in a laboratory aquatic system (*1*). Booij et al. (*20*) revealed the equivalent efficiency of SPMD and low density polyethylene membrane alone in sampling polychlorinated biphenyls (PCBs), PAHs, and chlorobenzenes under controlled conditions, indicating the role of triolein in the exchange kinetics was insignificant, particularly for compounds with log $K_{ow} > 6$. The importance of the polyethylene membrane as a solute reservoir in a three compartment model (water, polyethylene and triolein) has been mentioned by Gale (*21*). Additionally, the sampling rates of SPMD and the single layered polyethylene membrane sampler were not significantly different for compounds with log K_{ow} 4-7.5 at three different temperatures (*22*). Unlike the triolein impurities, analytical interferences from polyethylene membrane (i.e., polyethylene oligomers or polyethylene waxes) are readily removed by size exclusion chromatography (*18*). Therefore preparation and sample extraction of triolein-free low density polyethylene lay flat tubing (LFT) are less laborious and less expensive.

The triolein-free low density polyethylene membrane lay flat tubing (LFT) would be an alternative passive sampling device for monitoring exposure and assessment of contaminants. This simpler and less expensive sampling device would reduce the complexity of SPMD kinetic modeling and sample extraction, but provide the same benefits. However, there are some concerns for use of LFT. It was noted that the polyethylene membrane without

triolein appeared to be more subject to biofouling and it potentially reached equilibrium faster than SPMD because of its smaller sorption capacity (*20, 21*). We suspected that LFT would not reach equilibrium at ambient environmental concentrations of hydrophobic organic contaminants, suggesting the applicability of this simplified device. Also, information on the kinetic phase of chemical uptake during field deployment could be obtained by using permeability/performance reference compounds (PRCs) as an *in situ* calibration approach (*20, 23*). PRCs are analytical non-interfering compounds with moderate to relatively high fugacity from PSDs, which are added to the PSD prior to membrane enclosure(*23*). Because both the uptake and the dissipation of organic chemicals are controlled by the same molecular processes, *in situ* dissipation rates of PRCs would allow for estimating *in situ* PSD uptake rates (*20, 23*). For SPMD, triolein also served as a handy carrier for PRCs. A different approach needs to be developed for a triolein-free polyethylene membrane. Booij et al. (*24*) has developed spiking method based on equilibration of polyethylene membrane in 80/20 (v/v) methanol/water solution of PAHs and PCBs with log K_{ow} 3.9-7.7. However, variations in both the amount and the evaporation rates of the solvent that stuck to the membrane were sometimes problematic (*24*) and the membrane-solution partitioning coefficient was required. A suitable spiking method is thus needed.

Although the feasibility of LFT as an alternative passive sampler in aquatic systems has been mentioned in a few studies(*20-22*), its field application and demonstration were very limited (*25*). Further study of LFT as an alternative approach for passive sampling technique is worth the effort due to its comparable efficiency to SPMD and lack of interference from the triolein impurities. Development of LFT in both theoretical and experimental work as well as field study should be explored to promote a more complete understanding of its sampling mechanism and its applicability in the field.

In this study we evaluated the use of LFT as an alternative device for monitoring bioavailable hydrophobic organic contaminants in surface water and compared to to SPMD. A method for spiking PRCs into LFT was developed and their recoveries were tested in the laboratory under flowing water and in the field environment. LFT and SPMD were deployed side by side as a paired study in a contaminated harbor site in the lower Willamette River at Portland Harbor, Oregon. LFT sampling rates for selected polycyclic aromatic hydrocarbons

(PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides were estimated. Field derived data demonstrated the effectiveness and reliability of LFT for environmental monitoring.

Materials and Methods

Passive Sampling Devices. Low density polyethylene membrane lay flat tubing (LFT) (Barefoot®) was purchased from Brentwood Plastic, Inc (St. Louis, MO, USA). The LFT was free of additives. The wall thickness of LFT was approximately 75-95 μm. Standard SPMDs were purchased from Environmental Sampling Technologies (EST, St. Joseph, MO). A standard size SPMD consists of a 91-106 cm segment of 2.5 cm wide low density polyethylene lay flat tubing having a wall thickness of 70-95 μm and a surface area of 450 cm^2, that contains 1 mL of ≥ 95% pure triolein (1,2,3-tri[cis-9-octadecenoyl]glycerol) as a thin film and has a total weight of 4.5 g (*15, 16*).

Chemicals and solvents. Standards of organochlorine pesticides (purities ≥ 98.5%) and PAHs (purities ≥ 99%) were from Chem Service, Inc. (West Chester, PA). Standard PCBs (purities ≥ 99%) were from AccuStandard (New Haven, CT). SPMDs were fortified with PCB 82, and dibenz[a,h]anthracene for use as PRCs. List of target analytes is present in Table 3.1. Certified reference material of PCB congeners and organochlorine pesticides in cod liver oil (SRM 1588a) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). SRM 1588a was used for method validity and accuracy purposes for the determination of PCBs and organochlorine pesticides in complex lipophilic matrices such as triolein. All solvents used were pesticide or Optima® grade from Fisher Scientific (Fairlawn, NJ).

Table 3.1 Selected physicochemical properties of target analytes

	chemical		MW	log K_{ow}	minimal box dimension (Å)			molecular volume ($Å^3$)	molar volume (cm^3/mol)
					length	breadth	depth		
PAHs (*26, 27*)	naphthalene	(NAP)	128	3.4	8.9	7.2	3.1	126.9	147.6
	acenaphthylene	(ACY)	152	4.1	8.8	8.4	3.1		165.7
	acenaphthene	(ACE)	154	3.9	8.8	8.1	3.2	148.8	173
	fluorene	(FLO)	166	4.2	11.1	7.2	3.1	160.4	188
	phenanthrene	(PHE)	178	4.6	11.5	7.7	3.1	169.5	199
	anthracene	(ANT)	178	4.5	11.7	7.2	3.1	170.3	197
	fluoranthene	(FLA)	202	5.2	10.7	9.0	3.1	187.7	217
	pyrene	(PYR)	202	5.2	11.4	9.5	3.1	186	214
	benz[a]anthracene	(BAA)	228	5.6	13.7	9.4	3.1	212.9	248
	chrysene	(CHR)	228	5.9	13.6	7.7	4.4	212.2	251
	benzo[a]pyrene	(BAP)	252	6.5	13.6	8.9	3.1	228.6	263
	benzo[b]fluoranthene	(BBF)	252	6.1	13.6	9.3	4.5	230.3	268.9
	benzo[k]fluoranthene	(BKF)	252	6.8	13.3	9.1	3.1	231.1	268.9
	benzo[g,h,i]perylene	(BPL)	276	7.1	11.5	10.2	3.1	244.3	277
	indeno[1,2,3-c,d]pyrene	(IPY)	276	6.6	13.2	10.0	3.1		NA
	dibenz[a,h]anthracene[a]	(DBA)	278	6.5	15.6	9.3	3.1	255.4	300
OC pesticides (*28, 29*)	p, p'-DDT		355	5.7	13.1	8.7	8.2		261.3
	p ,p'-DDD		320	6.1	13.0	9.5	7.2		246.4
	p, p'-DDE		318	6.0	13.5	8.6	5.6		243.1
	dieldrin		381	3.5	10.3	8.9	7.7		
	methoxychlor[a]		346	4.2	13.4	11.0	6.6		
	endrin[a]		381	5.5	10.2	8.8	8.8		
PCBs (*30, 31*)	- di-CBs	PCB 8[a]	223	5.1				234.9	226.4
	- tri-CBs	PCB 37	256	5.9					247.3
	- tetra-CBs	PCB 44	290	6.0				259.1	268.2
		PCB 49	290	6.1				262.8	268.2
		PCB 52	290	6.1	11.4	7.8	5.6	262.8	268.2
		PCB 60	290	6.3				265.2	268.2
		PCB 74	290	6.7	13.9	7.8	4.8		
		PCB 77	290	6.5				267.6	268.2
	- penta-CBs	PCB 82[a]	324	6.1					
		PCB 87	324	6.5				272.4	289.1
		PCB 99	324	6.6					289.1
		PCB 101	324	6.4	12.6	7.8	5.6	276.1	289.1
		PCB 105	324	6.0					289.1
		PCB 114	324	6.7					
		PCB 118	324	7.1					289.1
		PCB 126	324	6.9					
	- hexa-CBs	PCB 128	358	7.0				282.4	310
		PCB 138	358	6.7	13.9	7.8	5.6		310
		PCB 153	358	6.9	13.9	7.8	5.6	289.4	310
		PCB 156	358	7.2					310
		PCB 166	358	7.0					
		PCB 169	358	7.6				265.2	310
	- hepta-CBs	PCB 170	392	7.1					
		PCB 180	392	7.2					330.9
		PCB 183	392	7.2	13.9	8.8	5.6		
		PCB 187	392	7.2					330.9
		PCB 189	392	7.7					

[a] used as permeability/performance reference compounds (PRCs) in passive sampling devices

Preparation of LFT. LFT was pre-cleaned by solvent extraction to remove impurities. Briefly, the tubing was pre-extracted twice with dichloromethane (Optima®, Fisher Scientific, Fairlawn, NJ) using a soxhlet extractor. After the second extraction, the pre-solvent extracted LFTs were dried by pulling a vacuum through polyurethane foam plugs. The apparatus was left under vacuum for approximately 48-72 hr at room temperature or until dichloromethane was removed from LFT. After dried, LFT was stored in clean, sealed paint cans, and frozen (-20°C).

Pre-extracted LFTs were heat-sealed at a distance of 2 cm from one end of which had been folded to make a small loop. The fortification standard solution was pipetted into the other end of the tube. The LFT used in the laboratory experiment were fortified with 100 μL of 400 ng/mL of PCB 8, PCB 82, PCB 170 and endrin, 800 ng/mL of methoxychlor, and 20 μg/mL of dibenz[a,h]anthracene in toluene. The LFT used in the field exposure were fortified with 100 μL of the mixture of PCB 82 and endrin (each at 200 ng/mL) and dibenz[a,h]anthracene (20 μg/mL) in toluene. These compounds were used as PRCs. Air was removed by squeezing the solvent down the interior of the tube toward the seal by running the tube through fingers (gloved hands). The remaining air was forced from the tube through the open end and the tube was heat-sealed. The LFT constructed in the present study was 2.7 ± 0.05 cm wide, 100 ± 0.22 cm long, with a mass of 5.5 ± 0.11 g and a surface area of 540 cm^2. Fortified LFTs were individually mounted on stainless steel racks (EST, St. Joseph, MO) and stored in cleaned paint cans which were tightly sealed and stored at -20°C until deployment.

Evaluation of the spiking method of permeability/performance reference compounds (PRCs) in LFT. In a pilot experiment, three fortified LFTs mounted on stainless steel racks were placed in an aquarium glass tank (35 cm x 50 m x 30 cm) filled with flowing tap water at flow rate 41 ± 7 mL/s and water temperature at 17 ± 1 °C in darkness to prevent photodegradation of dibenz[a,h]anthracene. After 14 days, LFTs were sampled and cleaned with gloved hands, then rinsed in 1N HCl (for approximately 15 sec) (Trace metal grade, Fisher Chemical, Fairlawn, NJ), 18 MΩ·cm water, acetone (Pesticide grade, Fisher Scientific, Fairlawn, NJ), and isopropanol (Pesticide grade, Fisher Scientific, Fairlawn, NJ), respectively. LFTs were kept in clean glass amber jars and stored at -20 °C until analysis.

Unexposed fortified LFT, fortified LFT procedural blank, reagent blank, and fortified reagent blank accompanied the exposed LFTs and were processed and analyzed exactly as the exposed samples.

Demonstration of effectiveness and reliability of LFT for field deployment. LFTs were tested at a field site in the lower Willamette River at Portland Harbor which is listed as an impaired water body in the Federal National Priority List (NPL, so called a superfund site) (*32*). The harbor sediments have been contaminated with PCBs, DDTs, PAHs, dioxin/furans, and heavy metals (*33*). Fish from this area were contaminated with DDTs and PCBs (*34*). The Willamette River at Portland Harbor is deep, slow moving, and tidally influenced (*33*). The harbor generally has a low sediment transport capacity (*33*). Surface water runoff is determined by climate influence and varies according to season (*33*).

LFTs were deployed side by side with SPMDs at the lower Willamette River at Portland Harbor from River Mile 1 to 18.5 which contained the Portland Harbor superfund site (RM 3.5 to 9.5) and the McCormick and Baxter Creosoting Co. superfund site (RM 7 East). The eleven sampling sites were outside the main shipping channel and often near stream outlets of various upstream land uses including industrial, urban/residential, and undeveloped areas. Five individual PSDs were loaded in a flow-through stainless steel cage. Duplicate cages of LFTs and SPMDs were submerged approximately 10 ft from the river bottom and were suspended with an "anchor-cable-cage-cable-float" arrangement. PSDs were deployed for 21 days. The average water temperature ranged from 9 to 22 °C. The five PSDs were later combined for analysis. On retrieval, PSDs were cleaned by gently rubbing with gloved hands in on-site water and the series of solvents described above. Cleaned PSDs were kept in clean glass amber jars and transported on ice-packs. Samples were stored at -20°C until analysis.

Sample extraction and chemical analysis. PSD extraction and cleanup were based on established protocols and only modified slightly as necessary (*5, 15*). Briefly, PSDs were dialyzed in hexanes, 400 mL for 5 PSDs (Pesticide grade, Fisher Scientific, Fairlawn, NJ) for 18 hr, followed by a second dialysis with fresh hexanes for 6 hr. The combined dialysates were concentrated by rotary evaporation and a TurboVap® LV evaporator (Zymark®,

Hopkinton, MA). The samples were cleaned and fractionated using gel permeation chromatography (Waters® gel permeation chromatography cleanup system; Water® 515 HPLC pump, 717 Plus autosampler, Phenogel® column, 2487 dual λ absorbance detector, and fraction collector II) (Milford, MA) with dichloromethane (Opima®, Fisher Scientific, Fairlawn, NJ) as the mobile phase at the flow rate of 5 mL/min. Appropriate fractions were determined by analyzing standards and fortified samples. The appropriate fraction (14-20 min) was collected and split into two equal sub-fractions: 1) PCB and OC pesticides and 2) PAHs. Both fractions were separately subjected to volume reduction using a TurboVap® LV evaporator and solvent exchanged into iso-octane (Pesticide grade, Fisher Scientific, Fairlawn, NJ) for PCB and OC fraction and acetonitrile (HPLC grade, Fisher Scientific, Fairlawn, NJ) for PAH fraction with a final volume of 1 mL.

PCB congeners and OC pesticides were determined using GC-μECD (Agilent Technologies 6890N Network GC system) (Palo Alto, CA) dual capillary columns (db-xlb and db-17, J&W Scientific Inc., Agilent Technologies, Palo Alto, CA) /dual detectors. Injector and detector temperature were at 250 °C, and 350 °C, respectively. The GC system was operated with helium carrier gas and nitrogen makeup gas. The oven temperature program was as follows: 100 °C (1 min) and increased at 1.2 $^{\circ}Cmin^{-1}$ to 265 °C (2 min).

PAH detection and quantitation was performed on a Hewlett Packard HPLC series 1100 (Agilent Technologies, Palo Alto, CA) with dual detection by fluorescence and diode array, both with multiple wavelengths. The fluorescence detector had an excitation wavelength at 230 and emission wavelengths at 360, 410, and 460 nm; the diode array had detection signals at 254, 242, and 230 nm. Only three compounds, fluorene, acenaphthylene, and indenol (1,2,3-c,d) pyrene, were detected by diode array; the rest were detected with the fluorescence detector. The column was a Phenomenex Luna C18 with 3-μm particle size (Phenomenex, Torrance, CA). The instrument was run with a constant flow rate of 0.75 mL/min and a timed gradient for the acetonitrile/water eluent system. The time program ran at 40% acetonitrile for 10 min, was gradually ramped up to 70% acetonitrile for 15 min, and then ramped up to 90% acetonitrile for 19 min. The program was held at 90% acetonitrile for 3 min and then returned to 40%.

Field duplicates and field blanks (trip blank, field blanks, and field extraction blanks) accompanying the deployed passive sampling devices during deployment, retrieval, and transportation to the laboratory were included in every batch. These field blanks and laboratory control blanks (i.e., procedural blanks, laboratory PSD controls, fortified samples, and standard reference material) represented 30 to 40% of a sample set and were processed and analyzed exactly as the deployed samples. The method detection limits (MDL) were determined as three times the heights of coincident peaks observed for each compound in the laboratory PSD control. For those analytes having no coincident peak, the MDLs were set at a value equivalent to the lowest standard in the respective calibration curve.

None of the target analytes were identified in field blanks and laboratory control blanks. The average percent recoveries of fortified samples were as follows: PAHs 70 ± 20%, PCBs 66 ± 13%, p, p'-DDT 99 ± 20%, p, p'-DDD 85 ± 8%, p, p'-DDE 79 ± 7%, and dieldrin 75 ± 17%. The average percent recoveries in standard reference material were as follows: PCBs 96 ± 24%, p, p'-DDT 99 ± 21%, p, p'-DDD 108 ±14%, p, p'-DDE 79 ± 11% and dieldrin 114 ± 27%.

Data analysis. Data interpretation was performed using SPSS® Version 10.0.1(SPSS Inc., 1989-1999), Sigma Plot 2002 for Windows Version 8.0 (SPSS Inc., 1986-2001), and Microsoft® Office Excel 2003 (Microsoft Corporation, 1985-2003). Standard descriptive statistics, paired *t*-test, linear regression techniques were used. In the field exposure experiment, the efficiency of LFT to sequester hydrophobic contaminants was evaluated. The efficiency of two PSDs was analyzed by comparing amounts of contaminants taken up per unit mass of LFT and SPMD using paired samples *t*-test. Relationships of LFT concentrations, SPMD concentrations, and physico-chemical parameters for PAHs, PCBs, and organochlorine pesticides were examined by linear regression approach.

Results and Discussion

Evaluation of the spiking method of permeability/performance reference compounds (PRCs) in LFT. In the laboratory study with LFT, LFTs were spiked with

selected PAHs, PCBs, and organochlorine pesticides in a small volume of toluene (100 μL). These compounds were used as PRCs (Table 3.2). Averaged recoveries of PRCs in unexposed LFTs were ≥ 75% except only for PCB 8 (2, 4'-dichlorobiphenyls) (log K_{ow} = 5.1) which yielded an averaged recovery of 62% (Table 3.2). PCB 8, a two chlorine substituted congener, has a relatively high vapor pressure (6.9 x 10^{-2} Pa) (*30*) compared to other target contaminants. It is plausible that volatilization of PCB 8 during sample extraction and analysis could contribute to its loss. In general, the percentage recoveries of unexposed LFT procedural control samples ≥ 75% indicated the dissipation of spiked compounds during sample preparation, storage and handling, and extraction was negligible. The spiked compound integrity was maintained until analysis. In addition, a stability test of spiked LFTs that were kept in closed amber jars at room temperature (22-25°C) for three weeks yielded an average recovery rate of 89% ± 11%. No significant loss of spiked compounds in the stability test suggested loss of sample integrity during transport or storage under 22-25°C would be negligible.

Table 3.2 Percent recoveries of permeability/performance reference compounds using simple spiking method in a small volume of toluene.

permeability/performance reference compound (PRC)	percent recovery (%)		PRC clearance rate k_{eprc} (d^{-1})
	exposure under flowing water (n=3)	unexposed laboratory control (n=9)	
PCB 8 (log K_{ow} = 5.1)	26 ± 8.1	62 ± 11	0.06
PCB 82 (log K_{ow} = 6.1)	67 ± 2.5	75 ± 9.1	0.008
PCB 170 (log K_{ow} = 7.1)	86 ± 4.0	87 ± 10	0.0008
endrin (log K_{ow} = 5.5)	98 ± 7.1	110 ± 20	0.008
methoxychlor (log K_{ow} = 4.2)	53 ± 16	114 ± 15[a]	0.05
dibenz [a,h] anthracene (log K_{ow} = 6.5)	91 ± 4.2	93 ± 13[a]	0.002

[a] n = 2

According to PRC approach, measuring the PRC loss over the exposure period provides an *in situ* clearance rate constant of PRC (k_{eprc}) (*23*),

$$k_{eprc} = ln\,(C_{PSD\text{-}0} / C_{PSD}) \bullet t^{-1} \quad (1)$$

where $C_{PSD\text{-}0}$ is the initial concentration of the PRC and C_{PSD} is the concentration of PRC remaining in the PSD following exposure, and t is exposure time in days. Comparison of the $k_{eprc\text{-}field}$ derived from the field exposed PSD to the $k_{eprc\text{-}lab}$ measured in calibration exposure ($k_{eprc\text{-}field} / k_{eprc\text{-}lab}$) can serve as an indicator of differences in the exposure conditions or the effect of environmental variables on PSD sampling (*23*).

In a two-week laboratory flowing water exposure study, $k_{eprc\text{-}lab}$ for compounds with log $K_{ow} \geq 5.5$ were less than 0.008 d^{-1} (Table 3.2). This finding confirms that polyethylene membrane without lipid reservoir (i.e., triolein) has the capacity to retain hydrophobic organic compounds by diffusive process in the polymer (*21*). The simple spiking method of PRCs in a small volume of toluene was effective and valuable for further development of LFT application. The result also suggests spiking with a small volume of other non-aqueous organic solvents such as iso-octane is applicable. This method is simple, inexpensive and provides the same benefit of a permeability/ performance reference compound approach.

Demonstration of effectiveness and reliability of LFT for field deployment. As part of our ongoing project for evaluation of seasonal bioavailability of contaminants in surface water at the lower Willamette River at Portland Harbor, Oregon (*35*), passive sampling devices were deployed for a period of 21 days. LFTs and SPMDs were deployed side by side as the paired study at thirteen sampling sites in three different conditions; average water temperature 9 ± 1°C /average river flow 14000 ± 4800 ft^3/s, average water temperature 22 ± 1°C /average river flow 8300 ± 390 ft^3/s, and average water temperature 22 ± 0.3 °C /average river flow 10000 ± 1700 ft^3/s. The total of 74 composite samples (each sample was a composite of 5 PSDs) of LFT and SPMD were compared. PCB 82, endrin and dibenz[a,h]anthracene were used as PRCs in both LFTs and SPMDs to account for the effects of environmental conditions on the device uptake rate.

Evaluation of LFT following field deployment. The outer surface of the body of LFTs and SPMDs, following each deployment period, was covered in a thin film of silt. It appeared that the samplers deployed under condition of water temperature 22°C / river flow 8300 ft^3/s in summer had slightly more fouling as compared to the other two conditions. However, in all cases the degrees of fouling on the exterior surface of LFTs and SPMDs which were deployed for 21 days were not significantly noticeably different. This observation differs from previous observations in which triolein-free SPMD was reported to be more subject to biofouling than SPMD (*20, 21*). After 21-day deployment of each sampling event, the total of 185 LFTs were not observed to have more biofouling than SPMD. The membranes of LFTs and SPMDs appeared to be free from biofouling and algae growth. Heavy biofouling was reported to reduce the permeability of diffusive-limiting membrane of SPMDs, resulting in lowering the sampling rate by as much as 30% for phenanthrene (log K_{ow} = 4.6) (*36*). Additionally, it was noticed that the membranes of LFTs after deployment were slightly more rigid than the membrane of SPMDs. The lack of rigidity of triolein-containing SPMD is plausibly due, in part, to the formation of a thin film of triolein derivatives or triolein impurities on the membrane exterior surface which might affect the contaminant uptake mechanism of SPMD.

Evaluation of the influence of field condition on contaminant uptake kinetic of LFT. Both uptake rate and clearance rate of hydrophobic contaminants in the passive sampler are governed by the same molecular processes (*21*). It has been proposed that any change in the uptake rate of contaminants to the passive sampler should be reflected by a change in the clearance rate from the passive sampler (*20, 23*). The device uptake rates may change due to changes in temperature, flow velocity of the surrounding water, and biofouling on the membrane surface during field exposure (*23*). The use of PRCs as an independent measure for the exchange kinetics between the passive samplers and water allows for device uptake rate adjustment for these field variables (*20, 23*). The ratio of the clearance rate constant ($k_{eprc\text{-}field}$) in the field and in the laboratory ($k_{eprc\text{-}lab}$) can serve as an indicator to differences in the environmental conditions. In addition, the information on the kinetic phase of contaminant uptake under field exposure can be obtained from the clearance of PRCs in the field (*20*). It has been suggested that the exchange kinetics are under aqueous boundary layer control for compounds with log K_{ow} 4.4-8.0 and under polyethylene membrane control for

compounds with log K_{ow} < 4.4 (*23*). However, it has been proposed that the use of PRCs with log K_{ow} 4.4-5.5 was also applicable to the target analytes under membrane control (*23*). Thus, the range of physico-chemical properties of PRCs used in this study was representative of all target analytes.

The PRC approach was used in both LFTs and SPMDs in the present study. Table 3.3 presents percent recoveries and clearance rates ($k_{eprc\text{-}field}$) of PRCs in LFTs and SPMDs which were paired-deployed under two different conditions. The PRC comparison study was done at 22 °C. Booij et al. (*24*) suggested complete dissipation of a PRC with log K_{ow} of 5 indicating all analytes with similar and lower log K_{ow} had attained equilibrium phase. In contrast, no dissipation of a PRC with log K_{ow} of 6 indicating all analytes with similar and higher log K_{ow} were in the linear uptake phase (*24*). Significant retention of PRCs with log K_{ow} of 5.5 to 6.5 in both LFTs and SPMDs (Table 3.3) suggested linear uptake phase for all target analytes could be assumed. Small deviation of the PRC recovery rates within each condition indicated insignificant variation from analyses or site-specific condition variation among sampling sites. Contaminant clearance times by LFT and SPMD were similar. The mean PRC clearance rate ($k_{eprc\text{-}field}$) in LFT and SPMD was not different (0.02 d^{-1} for LFT and SPMD at water temperature 22 °C, river flow 8300 ft^3/s; and 0.02 d^{-1} for LFT and 0.01 d^{-1} for SPMD at water temperature 22 °C, river flow 10000 ft^3/s). Similar clearance time indicated for a 21-day field exposure for both LFT and SPMD were governed by the same exchange kinetics.

PCB 82, endrin, and dibenz [a,h] anthracene clearance rates in the LFTs were not different between the two conditions (Table 3.3A and 3.3B). In contrast, the clearance rates for dibenz [a,h] anthracene in SPMDs were somewhat different between the two conditions. This finding suggests the exchange kinetics controlling more hydrophobic compounds such as dibenz [a,h] anthracene (log K_{ow} = 6.5) in LFTs is more robust to the changes in environmental condition than in SPMDs.

Table 3.3 Percent recoveries and clearance rates of permeability/performance reference compounds in the field exposed polyethylene lay flat tubing (LFT) and semipermeable membrane device (SPMD) under different field conditions.

A) Average water temperature 22°C, average river flow 8300 ft^3/s,

permeability/performance reference compound (PRC)	percent recovery (%)				PRC clearance rate k_{eprc} (d^{-1})		$k_{eprc\text{-}field}/k_{eprc\text{-}lab}$
	field sample (n=13)[a]		laboratory and field control (n=7)				
	LFT	SPMD	LFT	SPMD	LFT	SPMD	LFT
PCB 82 (log K_{ow} = 6.1)	62 ± 2.2	50 ± 3.0	81 ± 8.2	80 ± 5.9	0.01	0.02	1.25
endrin (log K_{ow} = 5.1)	41 ± 4.9	64 ± 4.9	93 ± 24	80 ± 10	0.04	0.01	5
dibenz[a,h]anthracene (log K_{ow} = 6.5)	84 ± 6.2	52 ± 13	92 ± 7.2	94 ± 4.2	0.004	0.03	2

[a] each sample was a composite of 5 PSD

B) Average water temperature 22°C, average river flow 10000 ft^3/s.

permeability/performance reference compound (PRC)	percent recovery (%)				PRC clearance rate k_{eprc} (d^{-1})		$k_{eprc\text{-}field}/k_{eprc\text{-}lab}$
	field sample (n=12)[a]		laboratory and field control (n=7)				
	LFT	SPMD	LFT	SPMD	LFT	SPMD	LFT
PCB 82 (log K_{ow} = 6.1)	65 ± 7.8	54 ± 6.0	73 ± 3.4	85 ± 8.9	0.006	0.02	0.75
endrin (log K_{ow} = 5.1)	33 ± 7.7	89 ± 11	87 ± 4.7	106 ± 15	0.04	0.008	5
dibenz[a,h]anthracene (log K_{ow} = 6.5)	85 ± 4.4	65 ± 7.6	92 ± 8.5	100 ± 4.8	0.004	0.002	2

[a] each sample was a composite of 5 PSD

Clearance rate ratios of PRCs between field and laboratory exposure in LFTs were not different either of the conditions tested (Table 3.3A and 3.3B). This suggested the increase of river flow from 8300 ft^3/s to 10000 ft^3/s or 20% difference did not significantly affect on the contaminant uptake kinetics of LFTs. The similar clearance rates of PRCs from Table 3.3A and 3.3B indicated the biofouling effect and river flow variation between the two conditions were negligible.

Although data for PRCs were not available for the sampling event of water temperature 9°C, a temperature effect can be expected (*22, 27, 29, 37*). The contaminant

uptake rate by polyethylene membrane is dependent on temperature in both aqueous boundary phase control and membrane phase control (*16*). Temperature can affect molecular diffusivity (*38*). Elevated temperatures result in more Brownian movement and elevated temperature media are less densely packed so that diffusivity of chemicals through them is facilitated. Evidence of the temperature effect on polyethylene membrane uptake rates has been documented (*22, 27, 29, 37*). For compounds under aqueous boundary control (log K_{ow} $\geq$ 4.4) (*22*) , the effect of a 13°C temperature change (from 9°C to 22°C) on aqueous diffusivity can be estimated from the ratio of diffusion coefficient in water (D_w) at 9°C and 22°C from the Stroke-Einstein relation and the equation by Hayduk and Laudie (*38*). A 1.5-fold increase in D_w was computed for a 13°C increase in temperature (D_w @ 22°C/ D_w @ 9°C = 1.5). It is noted that this calculation could not apply to the membrane boundary layer controls (i.e., polyethylene membrane). However, temperature increase in the chemical diffusivity in polyethylene membrane can be expected as there is increased polymer chain movement within the membrane due to an elevated temperature. Any temperature increase in the aqueous diffusivity and the membrane diffusivity should cause a corresponding increase in the uptake rate by LFT. Because contaminant partitioning and diffusion occurred at both aqueous boundary phase and polyethylene membrane layer, the uptake rates for compound with log $K_{ow} \geq 4.4$ are expected to increase at least 1.5 fold when water temperature changes from 9°C to 22°C.

Comparison of contaminant accumulation in LFT and SPMD. The paired LFT and SPMD field exposure study demonstrated that LFT accumulated PAHs, PCBs, and organochlorine pesticides as effectively as SPMD (Figure 3.1). Linear correlation between contaminants concentrations in LFT and in SPMD (amounts of contaminants accumulated per mass of PSD) (ng/g) indicated both devices functioned similarly. The capacity of LFT and SPMD to accumulate PAHs, PCBs, and organochlorine pesticides was approximately equivalent as expected from their similar surface areas. Lack of triolein as an ultimate reservoir in the device did not affect the capacity of LFT to accumulate hydrophobic organic contaminants under realistic exposure conditions. It has been hypothesized that a polyethylene membrane-only sampler reached equilibrium faster than triolein filled sampler (i.e., SPMD) because of their smaller sorption capacity, making it a poor choice for integrative long term monitoring (*21, 22*). Our study was conducted at a superfund site where

the river system has been contaminated with a wide range of contaminants. The result from our study indicated that LFT did not reach equilibrium state during a 21-day field exposure at a highly contaminated system. This finding indicated LFT performed at least as well as SPMD within the environmentally observed ranges of PAHs, PCBs, and organochlorine pesticides.

Concentrations of contaminants accumulated in LFT and SPMD at each sampling site for each deployment were statistically compared (a paired *t*-test, two-sided *p*-value ≤ 0.05 was considered significantly different). The mean concentrations for p,p'-DDT, p,p'-DDD, p,p'-DDE, and ΣPCBs (sum of 25 individual PCB congeners) in LFT were higher than in SPMD in all three different conditions. The congeners with log K_{ow} ranging from 6.0 to 7.0 were more concentrated in LFT than in SPMD. No statistical differences were observed between the concentrations in LFT and in SPMD for the congeners with log $K_{ow} < 6.0$ or > 7.0. The mean concentration of dieldrin, by contrast, was higher in the SPMD. No significant difference was observed for the mean concentration for ΣPAHs (sum of 15 priority pollutant PAHs). However, the mean concentrations of three-ring PAHs with log $K_{ow} < 4.5$ were higher in the SPMD. On the other hand, the mean concentrations of PAHs with log $K_{ow} \geq 6.0$ were higher in LFT. No differences were observed between the concentrations in LFT and in SPMD for PAHs with log K_{ow} 4.5-5.5.

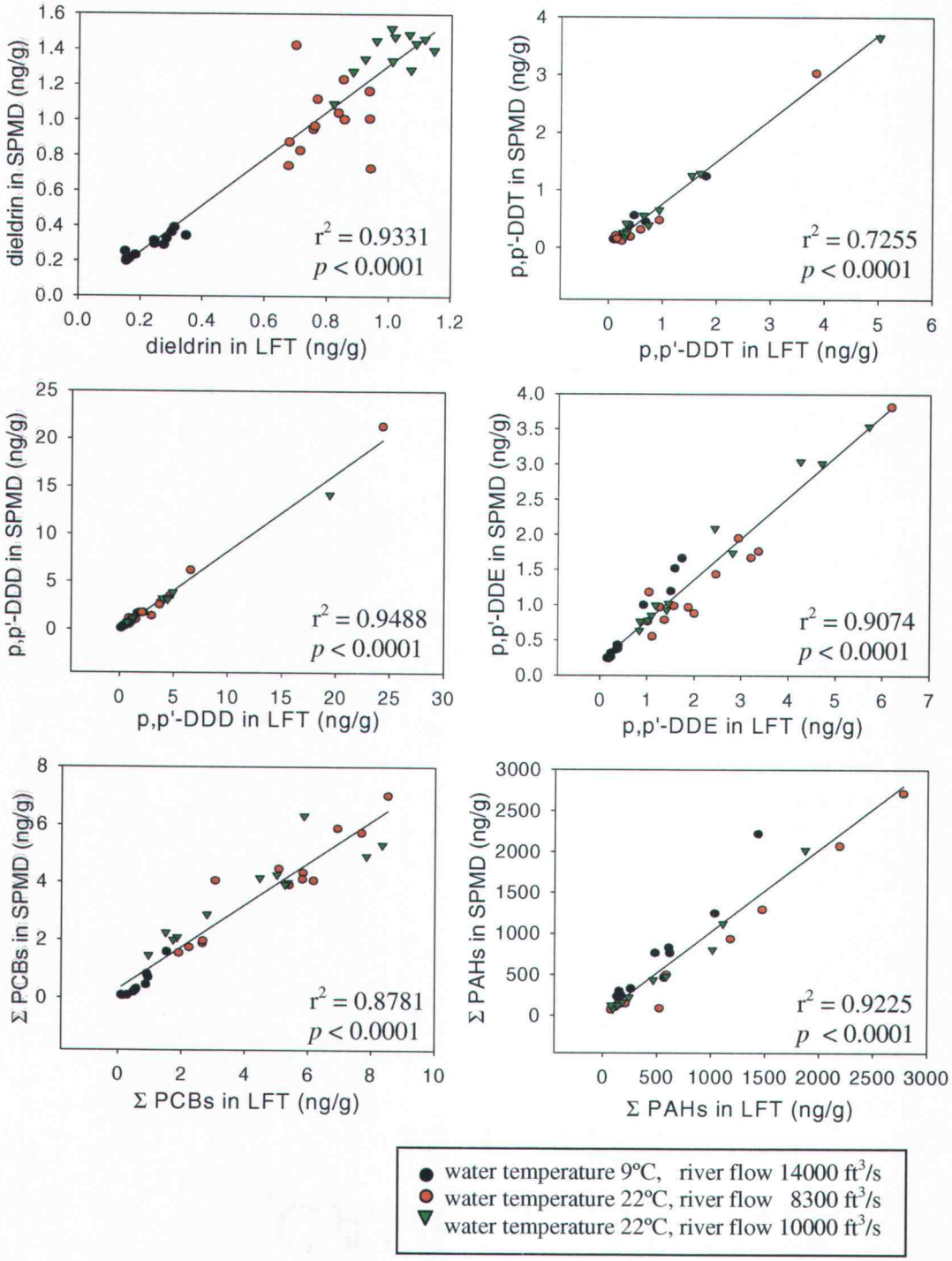

Figure 3.1 Relationship of contaminant concentrations in the triolein-free polyethylene lay flat tubing (LFT) and in semipermeable membrane device (SPMD). Linear correlation suggests both devices are governed by the same uptake model and function similarly. This indicates LFT can accumulate the selected contaminants as effectively as SPMD regardless of field variations.

Spatial distributions of contaminants accumulated in LFT were compared to those in SPMD. Figure 3.2 is an example of three deployments during 2003 to 2004. This 21-day exposure was taken during a period of water temperature 22 °C, river flow 8300 ft^3/s in summer of 2004. The spatial patterns for organochlorine pesticides and ΣPAHs in polyethylene lay flat tubing were identical to the patterns in SPMD. The spatial patterns for Σ PCBs in the two passive samplers were only slightly different at some sampling sites. This is mostly likely due to SPMD PCB concentrations near detection limits. A small variation between the PCB distributions in LFT and in SPMD at those sites is less significant especially when one considers the close resemblance of contaminants at the other sites. Compositions of PAHs and PCBs accumulated by LFT and SPMD from side by side field deployment were also very similar (Figure 3.3). The field exposure demonstrated the congener distributions and total concentrations were strongly correlated between the two sampling devices.

The LFT/SPMD concentration ratio was independent of temperature and flow regime in this study. The LFT/SPMD concentration ratio versus log K_{ow} (Figure 3.4) shows that for the forty-five contaminants tested, twenty-three were found to uptake in LFT faster than in SPMD (below detection limit contaminants were excluded from the discussion). For contaminants with log $K_{ow} > 5.0$, except PCB 37 and PCB 187, the LFT/SPMD concentration ratio is ≥ 1. Only ten compounds, generally all with log $K_{ow} \leq 4.5$, were found to have LFT/SPMD concentration ratios < 1.0. This finding indicates the role of triolein is insignificant for a compound with log $K_{ow} \geq 5$. For a compound with less hydrophobicity, triolein had more effect on contaminant accumulation. The effect of triolein on the amount taken up was about a factor of two for a compound with log $K_{ow} \leq 4.5$. In a laboratory flow control study by Booij et al. (*20*) using four SPMDs without triolein, the ratios of amounts taken up by SPMD without triolein / with triolein were equal for compounds with log $K_{ow} >$ 6, and the effect of triolein on the uptake kinetics was as large as a factor of four for a compound with log $K_{ow} = 4$. Our field study results corroborated the laboratory exposure results by Booij (*20*), indicating the overall effectiveness and reproducibility of LFT for sampling hydrophobic contaminants.

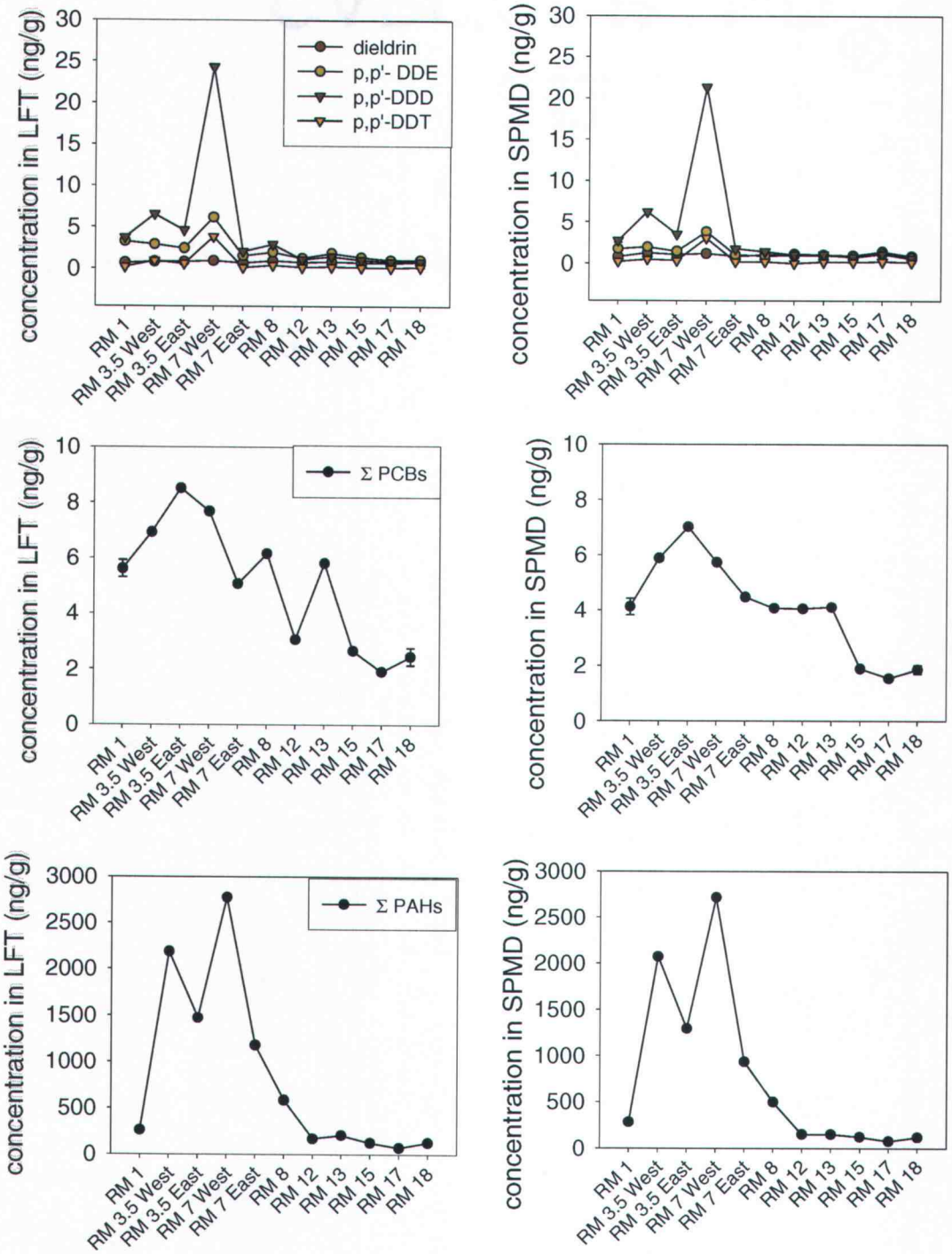

Figure 3.2 Comparison of spatial distributions of selected contaminants accumulated in the triolein-free polyethylene lay flat tubing (LFT) and in semipermeable membrane device (SPMD). LFT and SPMD were deployed side by side at the lower Willamette River at Portland Harbor, Oregon for 21 days in summer of 2004. Duplicated samples were deployed at River Mile 1 and River Mile 18.

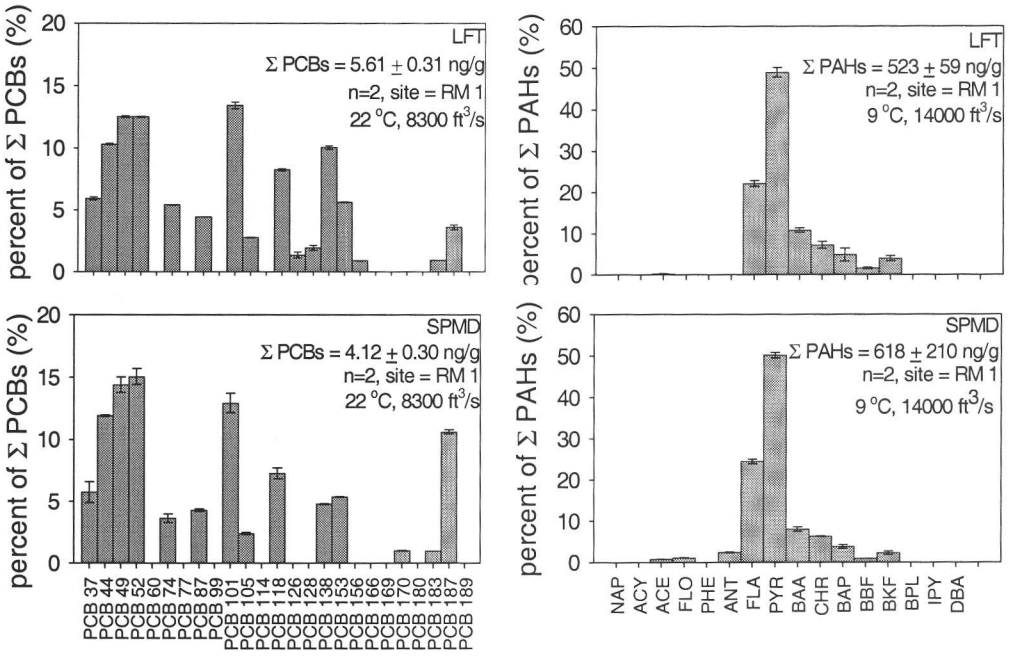

Figure 3.3 Percent composition of PCBs and PAHs (average ± 1sd) in the triolein-free polyethylene lay flat tubing (LFT) and semipermeable membrane device (SPMD) which were paired-deployed at the lower Willamette River at Portland Harbor, Oregon for 21 days. Total concentrations and field condition are noted in each panel. See Table 1 for abbreviations.

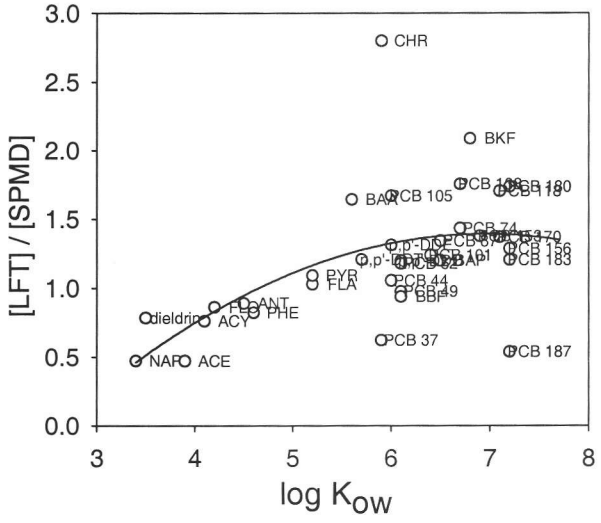

Figure 3.4 Average ratios of concentration of contaminants accumulated in the triolein-free polyethylene lay flat tubing (LFT) and in semipermeable membrane device (SPMD) as a function of octanol-water partitioning coefficient (log K_{ow}). See table 3.1 for abbreviation.

Estimation of LFT contaminant sampling rates. The present study demonstrates LFT is as efficient in sampling hydrophobic organic contaminants in water as is SPMD. The efficiency and reliability of LFT suggest the neutral lipid reservoir (i.e., triolein) is not always necessary. The LFT passive sampling device is theoretically less complex than SPMD. LFT can be modeled as a two compartment system while SPMD is a more complicated three-compartment system (*21*) Although less complex, the LFT passive sampling device maintains significant capacity to sample waterborne contaminants in a polluted eco-system. However, field application of LFT limited due to the lack of daily sampling rates for contaminants. The contaminant sampling rates allow back-calculation of aqueous contaminant concentrations. The input to the lack of established sampling rates for a wide array of contaminants is necessary to provide assessment to water quality standard, as well as temporal and spatial comparison.

The results in the present study, as well as, those by others (*21, 22*) indicate LFT or polyethylene membrane alone share the same contaminant uptake mechanisms with SPMD. Dissolved hydrophobic contaminants in water are transported by diffusion in the bulk aqueous phase to the aqueous diffusion film, then diffusion through the aqueous film and partition into the polyethylene membrane (*21*). Booij et al. (*22*) observed the difference between polyethylene membrane-water partitioning coefficients and SPMD-water partitioning coefficients from fifteen samples at three different temperatures were less than 0.6 log unit in the $4 < \log K_{ow} < 6$, suggesting the significant role of polyethylene membrane in contaminant uptake. There was no evidence of significant differences observed between sampling rates of SPMD and single-layered polyethylene membrane sampler in their study (*22*). The percent recoveries and clearance rates of PRC indicated the linear uptake model for LFT samplers and SPMDs deployed in this study. Estimated water concentration (C_w) by polyethylene containing passive sampling device (PSD) in the linear uptake model is obtained from the following equation (*16*)

$$C_w = C_{PSD} \bullet M_{PSD} \bullet R_s^{-1} \bullet t^{-1} \qquad (2)$$

where C_{PSD} is the concentration of the contaminant in the PSD (i.e., SPMD, LFT), M_{PSD} is the mass of PSD in grams, R_s is the sampling rate in liters per day of PSD, and t is the exposure time in days.

In the present study, LFT sampling rates for PAHs, PCBs, and organochlorine pesticides were estimated as a function of SPMD sampling rates under the same field conditions. When LFTs and SPMDs are pair-deployed, such as in this study, water concentration and other variables are the same for the paired deployment, leaving an R_S as the only unknown variable. Established SPMD sampling rates for PAHs, PCBs, and organochlorine pesticides at multiple temperatures have been well documented (*27, 29, 37, 39*). LFT sampling rates can be theoretically estimated from calibrated SPMD sampling rate.

$$R_{s\text{-}LFT} = C_{LFT} \cdot M_{LFT} \cdot R_{s\text{-}SPMD} \cdot C_{SPMD}^{-1} \cdot M_{SPMD}^{-1} \qquad (3)$$

Table 3.4 presents the LFT sampling rate estimates (R_s) at 9 °C and 22 °C. The LFT sampling rates were found to increase with increasing log K_{ow} and exposure temperature (Table 3.4 and Figure 3.5) as has been observed in SPMD (*22*). Large variation of LFT sampling rates was observed as log K_{ow} and molecular size increased (Figure 3.5). Membrane permeability limitation could be a factor contributing to this variation. Similar to biomembrane cavities, the diameters of polyethylene membrane transient cavities range up to 9-10 Å (*15*). This cavity size limitation impedes the diffusion of compounds with large molecular size or particle-bound contaminants through the polyethylene membrane. Contaminant molecules having at least two dimensions less than 10 Å can readily diffuse through the membrane cavities. Due to membrane cavity size limitation, reduction of LFT sampling rates for high K_{ow} compounds with breadths ≥ 9 Å, such as benz[a]anthracene and benzo[k]fluoranthene, were observed (Table 1 and 4, and Figure 5). On the other hand, the breadths of high K_{ow} PCB molecules are < 9 Å (Table 1) which allows them to diffuse through the membrane cavities. Also, the biphenyl bond of PCBs allows molecular conformation and rotation which may increase their membrane permeability. These differences in compound characteristics may contribute to large variation of LFT sampling rates for high K_{ow} compounds. Elevated temperatures may decrease the variation of LFT

sampling rates for high K_{ow} compound by increasing polymer chain movement within the membrane, which can facilitate chemical transport through the membrane.

Table 3.4 Contaminant sampling rates for triolein-free polyethylene lay flat tubing (LFT) estimated from field exposure to contaminated water at the lower Willamette River at Portland Harbor, Oregon for 21 days. LFT sampling rates were estimated from established SPMD sampling rates.

compound class	compound		molar volume (cm^3/mol)	log K_{ow}	sampling rate, L/d [a] 9 °C R_s	9 °C n	22 °C R_s	22 °C n
PAHs	naphthalene		147.6	3.4	1.3 ± 0.8	11	[b]	[b]
	acenaphthylene		165.7	4.1	2.2 ± 0.8	12	[b]	[b]
	acenaphthene		173	3.9	[b]	[b]	1.5 ± 0.5	13
	fluorene		188	4.2	3.4 ± 1.0	12	2.3 ± 0.6	6
	phenanthrene		199	4.6	4.3 ± 2.0	12	3.5 ± 0.8	7
	anthracene		197	4.5	3.5 ± 0.5	11	4.2 ± 0.9	8
	fluoranthene		217	5.2	3.7 ± 0.4	12	6.9 ± 1.1	22
	pyrene		214	5.2	4.5 ± 0.5	12	8.3 ± 0.9	21
	benz[a]anthracene		248	5.6	1.9 ± 0.3	2	6.4 ± 1.3	10
	chrysene		251	5.9	[b]	[b]	12.6 ± 4.9	13
	benzo[a]pyrene		263	6.5	[b]	[b]	12.9 ± 6.4	20
	benzo[b]fluoranthene		268.9	6.1	[b]	[b]	4.6 ± 1.4	12
	benzo[k]fluoranthene		268.9	6.8	2.4 ± 0.6	11	7.7 ± 3.8	7
OC pesticides	p,p'-DDT		261.3	5.7	3.5 ± 1.1	12	7.9 ± 2.6	24
	p,p'-DDD		246.4	6.1	2.4 ± 0.3	12	8.7 ± 1.8	25
	p,p'-DDE		243.1	6.0	1.6 ± 0.3	12	13.6 ± 3.0	25
	dieldrin			3.5	1.1 ± 0.1	12	4.5 ± 0.8	25
PCBs	- tri-CBs	PCB 37	247.3	5.9	[b]	[b]	7.1 ± 4.7	10
	- tetra-CBs	PCB 44	268.2	6.0	9.7 ± 1.5	4	13.7 ± 2.1	25
		PCB 49	268.2	6.1	6.0 ± 1.1	7	9.5 ± 2.6	24
		PCB 52	268.2	6.1	5.9 ± 1.3	12	11.5 ± 3.6	25
		PCB 74		6.7	6.3 ± 0.9	3	17.1 ± 3.3	10
	- penta-CBs	PCB 87	289.1	6.5	5.8	1	16.8 ± 2.9	15
		PCB 101	289.1	6.4	7.4 ± 1.3	6	17.3 ± 3.9	19
		PCB 105	289.1	6.0	[b]	[b]	11.5 ± 2.0	23
		PCB 118	289.1	7.1	4.1 ± 0.5	6	19.7 ± 3.0	2
	- hexa-CBs	PCB 138	310	6.7	7.3 ± 1.4	4	16.6 ± 8.6	8
		PCB 153	310	6.9	3.5 ± 1.0	5	15.0 ± 4.2	25
		PCB 156	310	7.2	[b]	[b]	12.4 ± 3.8	5
	- hepta-CBs	PCB 170		7.1	[b]	[b]	10.0 ± 3.1	8
		PCB 180	330.9	7.2	[b]	[b]	16.5 ± 1.8	6
		PCB 183		7.2	[b]	[b]	13.9 ± 4.7	4
		PCB 187	330.9	7.2	[b]	[b]	7.4 ± 1.7	14

values are average sampling rates; number in parenthesis are 1 SD

[a] sampling rate estimates for 1 triolein-free polyethylene lay flat tubing (LFT) sampler with a 100 cm long and 2.7 cm wide with a mass of 5.5 g and a surface area of 540 cm^2

[b] no value given due to concentration below detection limits

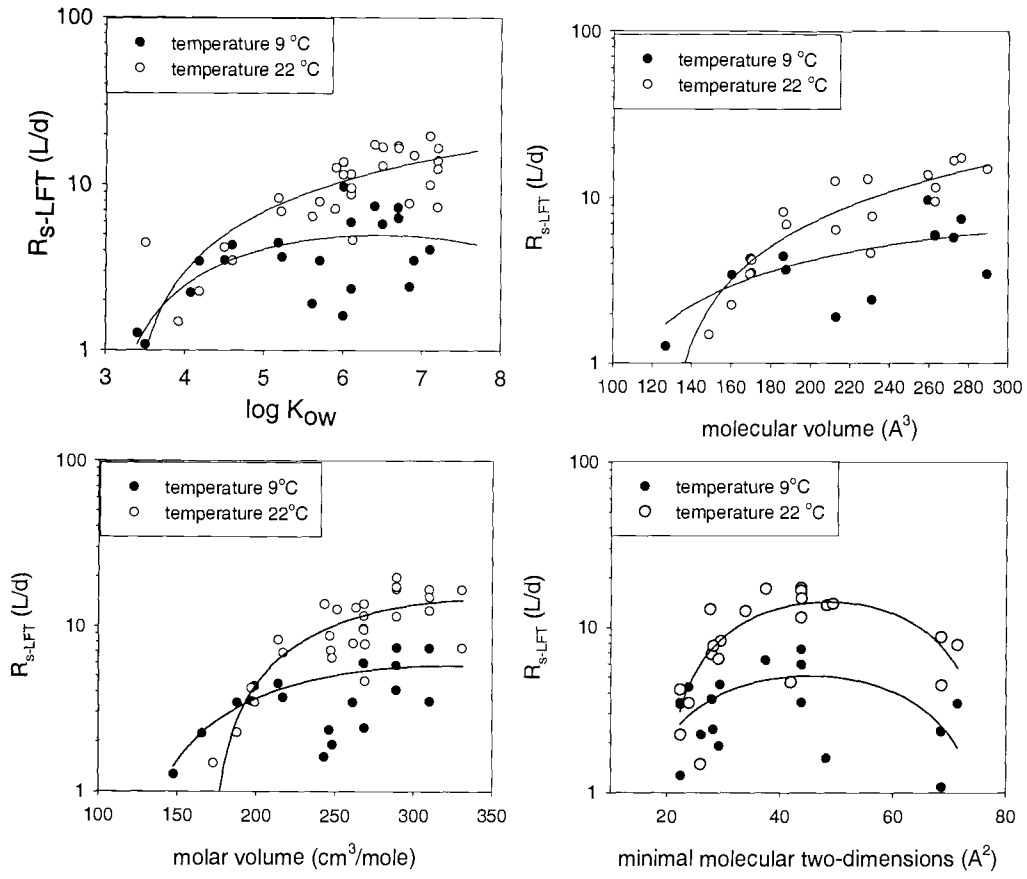

Figure 3.5 Sampling rate estimates ($R_{s\text{-}LFT}$) of a 540 cm^2 triolein-free polyethylene lay flat tubing (LFT) as a function of log K_{ow} ,molecular volume, molar volume, and minimal molecular two-dimension.

From Figure 3.5, the initial sharp increase of sampling rates with increasing log K_{ow} and molecular size is indicative of membrane-controlled uptake while the relative constancy of sampling rates at the large log K_{ow} and molecular or molar volume is indicative of aqueous boundary layer-controlled uptake. For membrane-controlled uptake, R_s is proportional to the product of the mass transfer coefficient for the polyethylene membrane (k_m) and the polyethylene membrane-water partition coefficient (K_{mw}) (*22*). Increasing molecular size and shape parameters can reduce membrane diffusivity (*40*). By contrast, since K_{mw} is hydrophobicity-related, it can be expected that increase in molecular weight, which is proportional to molar volume and log K_{ow}, would increase K_{mw}. For aqueous boundary layer-controlled uptake, the mass transfer coefficient for the aqueous boundary layer is a function of the kinetic viscosity of the water, and the compound's aqueous diffusion coefficient (D_w),

which is negatively related to molecular size (*40*). The difference in LFT sampling rates between compounds under aqueous boundary layer control can be preliminarily predicted from water diffusion coefficient (D_w). However, it was found that aqueous diffusivity alone cannot explain the difference in LFT sampling rates between compounds. For instance, within the same PCB homolog group which has equal chlorine numbers substituted and equal molar volume, LFT sampling rates for congeners with higher log K_{ow} tended to have higher LFT sampling rates. The result suggests although molecular size is a major factor controlling aqueous diffusivity for a compound under the aqueous boundary layer-controlled uptake, the hydrophobicity (log K_{ow}) that controls the chemical partitioning in the polyethylene membrane maintains a significant role in contaminant uptake by the LFT sampler and cannot be neglected.

According to the effect of aqueous diffusivity, a 1.5-fold increase in LFT sampling rates for compounds under aqueous boundary layer control (log $K_{ow} \geq 4.4$) was expected when water temperatures changed from 9°C to 22°C (see section *Evaluation of the influence of field condition on contaminant uptake kinetic of LFT*). The estimated LFT sampling rates for compounds with log K_{ow} ranging from 4.4 to 7.2 rose an average of 3.4-fold with the 13 °C temperature increase. The difference between the predicted and field exposure values indicated the temperature effect on aqueous diffusivity of a contaminant contributed 40% to an increase in LFT sampling rate for a temperature difference of 13 °C. This suggested that the other parameters involved in the uptake kinetic processes such as in the polyethylene membrane boundary layer (i.e., membrane diffusivity, log K_{ow}, and molecular size) are also significant contributors to an increase in LFT sampling rates for compounds under aqueous boundary layer control (log $K_{ow} \geq 4.4$).

Combining the effects of aqueous diffusivity, log K_{ow}, and molecular size on the LFT sampling rates, the LFT sampling rates can be modeled using a linear regression model, see Figure 3.6. Organochlorines and PAHs were separately modeled. Both models were statistically significant ($p < 0.05$), suggesting the LFT sampling rates can be predicted from the product of the aqueous diffusivity, log K_{ow}, and the minimum molecular two-dimensions of a contaminant. The fitting models for these two contaminant classes confirm the important roles of chemical diffusion in both polyethylene membrane (log K_{ow}, and minimal 2-

dimension) and aqueous boundary layer (aqueous diffusivity, molar volume). The LFT uptake rates for other PCB congeners or PAHs can possibly estimated using equations in Figure 3.6.

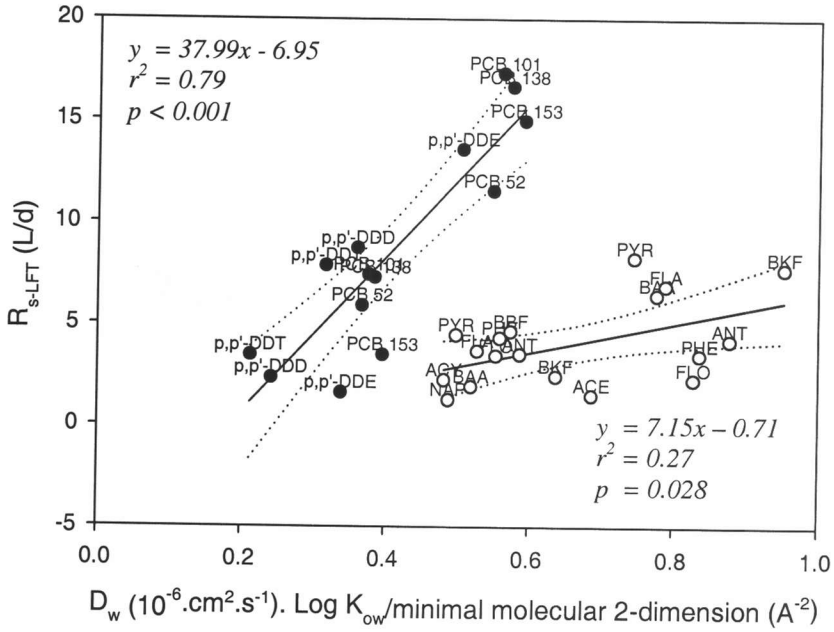

Figure 3.6 Sampling rate estimates of a 540 cm^2 triolein-free polyethylene lay flat tubing (LFT) sampler as a function of the product of aqueous diffusivity, log K_{ow} and minimum molecular two-dimension.

Another concern of using triolein-free polyethylene membrane as a passive sampling device is its relatively small sorption capacity due to lack of triolein (*21, 22*), subsequently the uptake phase would reach equilibrium during field deployment. In the present study, the linear relationship between the amounts of contaminants accumulated in LFT and SPMD and the results from PRC approach indicated both LFT and SPMD were in the linear uptake phase. In addition, times for the phases of polyethylene membrane sampler uptake can be estimated from (*16*)

$$t_{1/2} = -ln\ 0.5\ K_{ow}\ V_{PSD} / R_s \qquad (4)$$

where $t_{1/2}$ is the time required to accumulate 50% of the equilibrium concentration which essentially represents the linear region of uptake, V_{PSD} is the total volume of the PSD. Using LFT sampling rates in Table 3.4, the average deployment durations for compounds with $4.4 \leq \log K_{ow} \geq 7.7$ to maintain in the linear uptake phase during LFT deployment are 2,250 days at 9 °C (range from 29 to 9,810 days) and 1,280 days at 22 °C (range from 24 to 6,860 days. Evaluated together, our results confirm LFT sampler would not reach equilibrium during 21-day exposure at realistic environmental concentrations even at a highly contaminated site with a wide array of contaminants, such as Portland Harbor superfund site.

In summary, the triolein-free polyethylene lay flat tubing (LFT) showed the ability to detect the selected hydrophobic contaminants as efficient as the SPMD. Its sampling efficiency suggested the neutral lipid reservoir (triolein) present in SPMD was not always necessary. Also, biofouling was not a problem for its field application as compared to SPMD. This simpler device tended to accumulate compounds with high log K_{ow} faster than SPMD. Approaching equilibrium appeared not be a concern at realistic environmental concentrations for 21-day exposure. Spiking permeability/performance reference compounds can be achieved by using simple spiking method in a small volume of toluene. The successful determination of field derived data illustrated the effectiveness and reliability of the triolein-free polyethylene lay flat tubing for environmental monitoring. Its application increases the range of tools available to those involved in monitoring water quality. The method of choice for environmental monitoring will depend on the contaminant nature and environmentally relevant concentrations of contaminants as well as the objective of a study.

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CHAPTER 4

ENVIRONMENTAL STRESSES AND FISH DEFORMITIES IN THE WILLAMETTE RIVER: COMPARISON OF PERSISTENT BIOACCUMULATIVE TOXICANTS

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Abstract

In response to public concern related to the reports of a high incidence of skeletal deformities in fish at the Willamette River, Oregon from River Mile 26.5-55, an area known as Newberg Pool, a team of multidisciplinary scientists from Oregon State University collaborated to identify the cause(s) of skeleton deformities associated with Willamette River fish, with particular emphasis on the Newberg region. Our research group's effort was to compare and contrast water chemical-physical conditions and the bioavailable fractions of PCBs, DDTs, and dieldrin at the Newberg and Corvallis areas, where fish deformities were significantly lower, and determine whether these factors were likely causes of the deformities. *In situ* monitoring of river water quality coupled with *in situ* sampling and analysis of bioavailable PCBs, DDTs, and dieldrin by semipermeable membrane device (SPMD) were used. There were some differences in water quality parameters between the Newberg and Corvallis sampling sites. However, they did not readily explain the difference in deformity loads. Concentrations of bioavailable Σ PCBs, Σ DDTs, and dieldrin were generally below 50 pg/L and the concentration differences observed between sites were very small, with the mean concentration differences ranged from none to 27 pg/L. The results did not provide compelling support for the hypothesis that PCBs, DDTs and dieldrin present in surface water or differences in water quality parameters were a likely cause of the different deformity loads observed at Newberg versus Corvallis.

Introduction

In response to public concern related to the reports of a high incidence of skeletal deformities in fish at the Willamette River from River Mile 26.5-55 (an area known as Newberg) (*1-3*), a team of multidisciplinary scientists from Oregon State University collaborated to identify the cause(s) of skeleton deformities associated with Willamette River fish, with particular emphasis on the Newberg region (*4*). The incidence skeleton deformities in fish from the Newberg region had significantly greater deformity rates (56 %) than fish from the upper Willamette River (i.e., 2-5 % at Corvallis), see location in Figure 4.1 (*2*). Pre-caudal deformity load in northern pikeminnow showed a spatial pattern with a significantly higher incidence at Newberg area than the sampling sites at Newberg tributaries and the upper Willamette River(*3*). The high frequency of skeletal defects at the Newberg area and the gradual decline in the upstream and downstream direction suggested a local source as the cause of skeleton defects in this region. The collaborative study consisted of three main components as follows; investigation prevalence of skeleton deformities in juvenile fish from the Willamette River focusing on Newberg (high prevalence) and Corvallis (low prevalence) sites; comparison and contrast water physical and chemical conditions and distribution of selected chemical contaminants at these sites; laboratory toxicity experiments with complex chemical mixtures and other stressors from these sites. Together, these components provided a weight of evidence based empirical approach to identify the likely cause of skeletal deformities in Willamette fish. The final report has been published in *Environmental Science and Technology. Volume 39, No. 10, 2005, page 3495-3506, "Environmental stresses and skeletal deformities in fish from the Willamette River, Oregon"* (*4*), see Appendix E.

Our research group's effort focused on the distribution of persistent bioaccumulative toxicants in surface water. We compared water quality and potential for direct exposure to these chemical contaminants at the Newberg sampling site, north and south side of the area at River Mile 44 and 47, respectively, compared to the upper Willamette River sampling site at Corvallis (River Mile 135) and determined whether these factors were likely causes of the deformities. Inclusion of persistent bioaccumulative toxicants to be analyzed was based on in-depth review of the peer-review literatures. A wide variety of chemical, physical, and biological stressors have been associated with skeleton deformities in fish (*5-10*). This

chapter focuses on the distribution of chemical contaminants including polychlorinated biphenyls (PCBs), DDTs (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane and its metabolites) and dieldrin which have been reported to cause skeleton deformities by impairing developmental processes and bone formation (*6-8*). Smith and Cole (*6*) found embryo developing from eggs laid by adult winter flounder that were exposed to DDT showed a high incidence of vertebral deformities caused by bone erosion and hemorrhaging at the vertebral junctures. Olsson et al. (*7*) found offspring zebrafish following maternal exposure to certain PCB congeners showed craniofacial and posterior malformation. Skeletal deformities have also been linked to water quality problems, including low pH (*5*), low dissolved oxygen (*11*), and elevated temperature (*12*).

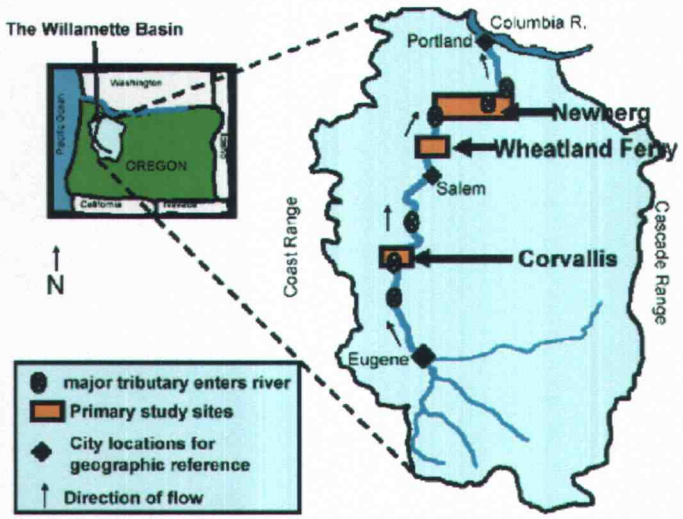

Figure 4.1 The Willamette Basin showing the general location and course of the Willamette River and primary study locations.

The main purpose of this work was to compare and contrast water chemical-physical conditions and the bioavailable fractions of PCBs, DDTs, and dieldrin at the Newberg and Corvallis and determine whether these factors were likely causes of the deformities. *In situ*

monitoring of river water quality coupled with *in situ* sampling and analysis of bioavailable PCBs, DDTs, and dieldrin by semipermeable membrane device (SPMD) were used. SPMD consists of polyethylene lay flat tubing containing a thin film of a neutral lipid, ≥ 95% pure triolein (1,2,3-tri[cis-9-octadecenoyl]glycerol) (*13*). It has been proposed that SPMD mimics key mechanisms of bioconcentration including diffusing through biomembranes and partitioning between organism lipid and the surrounding medium (*14*). During spawning/early fish development (mid May through July 2002 and 2003), the *in situ* water quality monitoring device (YSI 6920 SONDE; Yellow Springs, OH) and SPMD were deployed to continuously collect a set of physical/chemical data. In this way episodic events and other pulses of contaminants were captured. Temperature, dissolved oxygen, pH, specific conductivity, oxidation reduction potential, NH_4^+-N,and NO_3^--N concentrations were collected on an hourly basis during each 21-day sampling event.

Materials and Methods

Passive sampling device. Standard SPMDs were purchased from Environmental Sampling Technologies (EST, St. Joseph, MO). A standard size SPMD consists of a 91-106 cm segment of 2.5 cm wide low density polyethylene lay flat tubing having a wall thickness of 70-95 μm and a surface area of 450 cm^2, that contains 1 mL of ≥ 95% pure triolein as a thin film and has a total weight of 4.5 g (*14, 15*).

Chemicals and solvents. Standards of organochlorine pesticides (purities ≥ 98.5%) were from Chem Service (West Chester, PA). Standard polychlorinated biphenyls (PCBs) (purities ≥ 99%) were from AccuStandard (New Haven, CT). All solvents used were pesticide or Optima® grade from Fisher Scientific (Fairlawn, NJ).

Sample collection. The Willamette River sampling sites were chosen to facilitate investigation of spatial and seasonal bioavailable concentrations of organic contaminants at Newberg and Corvallis during May to July 2002 and 2003 (Figure 4.1.). Two sampling sites were located at Newberg; one on the south of the river (River Mile 47) and one a few miles downriver on the north side of the river (River Mile 44). Two sampling sites were located at

Corvallis at River Mile 135. SPMDs and YSI 6920 Sonde (YSI, Yellow Springs, OH) were deployed from May to July 2002 and 2003 to sample dissolved, bioavailable PCBs and organochlorine pesticides and to collect water chemistry parameters, respectively. Three 21-day sampling events were completed per year, one each in May, June, and July. The sampling coincided with critical fish life stages during spawning and early development. River depth and water chemistry parameters including temperature, dissolved oxygen, pH, specific conductivity, oxidation reduction potential, NH_4^+-N,and NO_3^--N concentrations were measured on an hourly basis by YSI 6920 Sonde.

Five individual SPMDs were in a flow-through stainless steel cage. Each cage was suspended 1 ft. from the river bottom with submerged with "anchor-cable-cage-cable-float" arrangement. The five SPMDs were later composited for analysis. On retrieval, SPMDs were cleaned by gently rubbing with gloved hands in on-site water, then rinsed in 1N HCl (approximately for 15 sec) (Trace metal grade, Fisher Chemical, Fairlawn, NJ), 18 MΩ.cm water, acetone (Pesticide grade, Fisher Scientific, Fairlawn, NJ), and isopropanol (Pesticide grade, Fisher Scientific, Fairlawn, NJ), respectively. Cleaned SPMDs were kept in clean glass amber jars and transported on ice-packs. Samples were stored at -20°C until analysis.

Sample extraction and chemical analysis. SPMD extraction and cleanup were described in detail in Chapter 2 and 3. Briefly, SPMDs were extracted by hexanes dialyses (Pesticide grade, Fisher Scientific, Fairlawn, NJ) in airtight amber glass jars. Sample volumes were reduced using rotary evaporation and TurboVap® LV evaporator (Zymark®, Hopkinton, MA). The samples were cleanup and fractionated using gel permeation chromatography (Waters® gel permeation chromatography cleanup system; Water® 515 HPLC pump, 717 Plus autosampler, Phenogel® column, 2487 dual λ absorbance detector, and fraction collector II) (Milford, MA) with dichloromethane (Opima®, Fisher Scientific, Fairlawn, NJ) as the mobile phase at the flow rate of 5 mL/min. Appropriate fractions were determined by analyzing standards and fortified samples. The fraction contained PCB and organochlorine pesticides was subjected to volume reduction using TurboVap® LV evaporator and solvent exchanged into iso-octane (Pesticide grade, Fisher Scientific, Fairlawn, NJ) and were analyzed using GC-μECD (Agilent Technologies 6890N Network

GC system) (Palo Alto, CA) dual capillary columns (db-xlb and db-17, J&W Scientific Inc., Agilent Technologies, Palo Alto, CA) /dual detectors.

Quality control. Field duplicates and field blanks accompanying the deployed passive sampling devices during deployment, retrieval, and transportation to the laboratory were included in each sampling event. The Corvallis site was designated as a field duplicate site. These field blanks and laboratory control blanks represented 30 to 40% of a sample set and were processed and analyzed exactly as the deployed samples. None of the target analytes were identified in field blanks and laboratory control blanks.

The percent recoveries of fortified samples were as follows: PCBs 57-110%, and organochlorine pesticides 56-72%, relative percent difference between field duplicates was ≤ 46%. PCB 170 and methoxychlor were used as permeability/performance reference compounds (PRCs), which were added to SPMD prior membrane enclosure (*16*). The use of PRCs as an independent measure for the exchange kinetics between SPMDs and water allows the device uptake rate adjustment for changes in temperature, flow velocity of the surrounding water, and biofouling on the membrane surface during field exposure (*16, 17*). The mean clearance rates of PCB 170 and methoxyclor indicated no significance loss during SPMD deployment. Biofouling or algae growth on SPMDs was nil to minimal in this study. No adjustment of sample concentrations for laboratory percent recoveries and percent loss in field was taken.

Data analysis. The basic theory and mathematical models required for estimation of analyte water concentrations from the concentrations in the SPMD have been described by Huckins et al (*15*). Estimates of water concentrations (C_W) from SPMD concentrations were calculated by the following equation (*15*),

$$C_W = C_{SPMD} \cdot M_{SPMD} \cdot R_s^{-1} \cdot t^{-1}$$

where C_{SPMD} is the concentration of the individual analyte in the SPMD, M_{SPMD} is the mass of SPMD in grams, R_s is the sampling rate of a standard 1-g triolein SPMD, and t is the time

in days. SPMD sampling rates (R_s) for PCBs and organochlorine pesticides at multiple temperatures have been established (*18-20*).

Data interpretation was performed using SPSS® Version 10.0.1(SPSS Inc., 1989-1999), Sigma Plot 2002 for Windows Version 8.0 (SPSS Inc., 1986-2001), and Microsoft® Office Excel 2003 (Microsoft Corporation, 1985-2003). Standard descriptive statistics and the Mann-Whitney test (nonparametric two-independent-samples tests) were used to evaluate differences between sampling sites and sampling year. Statistical analyses were considered significant at $p \leq 0.05$.

Results and Discussions

Water chemistry characterization. Temperature, dissolved oxygen, pH, oxidation reduction potential, specific conductivity, NH_4^+-N,and NO_3^--N concentrations were collected hourly during all 21-day sampling events in May-July, 2002 and 2003 (Table 4.1). Discrete sampling with 4-second interval was also measured during SPMD deployment and retrieval and was reported when the 21-day data were unavailable. At each sampling site, some parameters showed spatial and temporal variation while others did not.

The temperature patterns at Newberg and Corvallis sampling sites were similar for both years and were generally 14 ºC in May and rose to 20 ºC by the end of the sampling period. The average river temperature at Newberg sampling site was generally 1-2 ºC higher than at Corvallis. Diurnal temperature variation was similar at both sites (1-2 ºC or less). Dissolved oxygen probe tended to drift after about 10 days of deployment in some sampling events. In that case the data after 10 days were excluded from the analysis. The dissolve oxygen pattern was similar at both sites. A slight decrease in dissolved oxygen was seen from May to July in both 2002 and 2003, as can be expected as water temperature increased.

Table 4.1 Spatial and temporal variations of water quality parameters at Newberg (River Mile 44-47) versus Corvallis (River Mile 135) sampling sites on the Willamette River, Oregon.

water quality parameter	year	month	Newberg	Corvallis
temperature (°C)	2002	May	14 ± 1.2	13 ± 0.93
		June	16 ± 1.3	16 ± 1.3
		July	19 ± 1.3	19 ± 1.5
	2003	May	14 ± 1.7	15 ± 0.0 [a]
		June	17 ± 1.1	16 ± 1.3
		July	20 ± 2.0	19 ± 1.9
dissolved oxygen (mg/L)	2002	May	12 ± 0.49	11 ± 0.52
		June	9.9 ± 0.61	9.2 ± 1.4
		July	9.6 ± 0.35	9.5 ± 0.39
	2003	May	12 ± 0.28 [a]	10 ± 0.0 [a]
		June	10 ± 0.41	11 ± 0.8
		July	9.7 ± 0.41	9.6 ± 1.6
pH	2002	May	7.5 ± 0.06	7.6 ± 0.16
		June	7.5 ± 0.12	7.6 ± 0.26
		July	7.3 ± 0.04	7.4 ± 0.01
	2003	May	7.4 ± 0.12	7.4 ± 0.0
		June	7.4 ± 0.16	7.7 ± 0.4
		July	7.2 ± 0.10	7.4 ± 0.1
oxidation-reduction potential or ORP (mV)	2002	May	260 ± 26 [b]	370 ± 16 [b]
		June	120 ± 13 [b]	340 ± 26 [b]
		July	290 ± 17 [b]	440 ± 33 [b]
	2003	May	270 ± 40 [b]	165 ± 2.3 [a]
		June	380 ± 8.7 [b]	290 ± 32 [b]
		July	440 ± 20 [b]	390 ± 36 [b]
specific conductivity (mS/cm)	2002	May	0.063 ± 0.001	0.060 ± 0.002
		June	0.057 ± 0.004	0.067 ± 0.004
		July	0.078 ± 0.004	0.075 ± 0.003
	2003	May	0.073 ± 0.002	0.064 ± 0.000 [a]
		June	0.060 ± 0.006	0.063 ± 0.004
		July	0.087 ± 0.002	0.061 ± 0.002
NH_4^+-N (mg/L)	2002	May	0.04 ± 0.01	0.05 ± 0.01
		June	0.04 ± 0.01	0.09 ± 0.01
		July	0.17 ± 0.03	0.16 ± 0.01
	2003	May	0.04 ± 0.01	0.10 ± 0.0 [a]
		June	0.10 ± 0.02	0.07 ± 0.02
		July	0.03 ± 0.01	0.02 ± 0.00
NO_3^--N (mg/L)	2002	May	NA	NA
		June	NA	NA
		July	0.86 ± 0.05 [a]	1.17 ± 0.16 [a]
	2003	May	2.8 ± 0.30 [a]	1.1 ± 0.1 [a]
		June	NA	NA
		July	0.36 ± 0.14 [a]	0.2 ± 0.0 [a]

[a] Continuous sampling data were not available. Discrete sampling data taken during SPMD deployment and retrieval were reported.

[b] ORP probe tended to drift after 7-10 days of deployment. Data after 7 data were excluded from the analysis.

NA- data not available

Maximum pH range, usually in late May to mid June was 7.4 to 8.6 at Corvallis sampling site, and 7.2 to 7.8 at Newberg sampling site. The daytime pH showed a strong spatial difference, with the Corvallis sampling site often 1+ pH units higher than the Newberg sampling site. By contrast, the night time pHs were similar at both sites. These large diurnal pH changes were seen in both years. There were small decreases in pH from 2002 to 2003. Although some deformities in fish have been linked to pH effects such as muscular and spinal deformities due to decalcification in white sucker held at pH 4.2 for over 4 weeks (5), we are unaware of any peer-reviewed literatures linking pH 7.2 to 8.6 conditions to skeleton deformities in fish.

The oxidation reduction potential (ORP) probe tended to drift after 7-10 days of deployment. Since all deployments were 21 days, data after 7 days were excluded from the analysis. In 2002, the ORP was lower at the Newberg sampling sites as compared to the Corvallis sampling site with the difference ranged from < 10% to a factor of 2. In contrast, the ORP at Newberg was higher than Corvallis in 2003. There was some evidence that ORP differed among years at the Newberg sampling sites. The ORP value is a direct reading of the activity of oxidizing and reducing agents in the water as they correspond to oxidation-reduction reactions. In general, there was no specific ORP pattern difference between Newberg and Corvallis sampling sites although the Newberg waters were observed more reducing than Corvallis waters in 2002. ORP is known to influence microbial growth, but it was not clear whether the difference in ORP observed would make Newberg fish more vulnerable to infection by microbial agents or more susceptible to deformities.

Specific conductivity was similar at the Newberg and Corvallis sampling sites for both years. A seasonal increase in NH_4^+-N concentrations was observed at both sites from May to early July, with Corvallis generally having higher NH_4^+-N than Newberg. The NO_3^--N probe was not robust. In 2002, drift generally occurred within 24 hours of deployment. In 2003, the probe failed within a few hours of deployment. The limited data indicated that NO_3^--N decreased from May to July, the concentrations were higher at the Newberg sampling sites as compared to the Corvallis sampling sites. Evaluated together, water chemistry parameters at both sites provided no compelling evidence to suggest that the differences in

these parameters were likely causes of the different fish deformity loads observed at Newberg versus Corvallis.

Bioavailable polychlorinated biphenyls (PCBs). In the present study, PCB analysis was based on a congener-specific approach; however, concentrations of all congeners were quite low and were below detection limits (0.71-4.4 pg/L depending on congeners) in many samples, so interpretation of results focused on ΣPCB concentrations (sum of 24 congeners). The bioavailable concentrations of ΣPCBs were generally very low at all sites (Table 4.2). During 2002 and 2003, biovailable ΣPCB were generally greater at Newberg sampling sites (Table 4.2). However, since most of the data were near or below detection limits, it was difficult to draw any firm conclusions. Bioavailable concentrations of ΣPCBs were approximately 500-fold lower than the national and the Oregon fresh water aquatic life criteria for ΣPCBs (0.014 μg/L) (*21, 22*) and they did not readily explain the difference in deformity load among sites.

Table 4.2 Mean concentrations (pg/L) of bioavailable organochlorines estimated from concentrations accumulated in semipermeable membrane devices (SPMDs) exposed for 21 days at the Willamette River at Newberg and Corvallis (2 sampling sites per location) in 2002 and 2003

chemical	spatial distribution						temporal distribution p-value	
	2002			2003				
	NP n=6	CV n=6	p-value	NP n=6	CV n=6	p-value	NP	CV
ΣPCBs[d]	13 ± 15	7.0 ± 11	0.39	15 ± 5.2	8.7 ± 4.0	0.015	1.000	0.394
ΣDDTs	49 ± 18	22 ± 28	0.18	48 ± 11	36 ± 4.2	0.065	0.485	0.240
p, p'-DDT[c]	10 ± 5.2	2.9 ± 4.6	0.041	11 ± 2.2	11 ± 0.99	0.394	1.000	0.004
p, p'-DDE[c]	16 ± 5.3	6.2 ± 7.6	0.041	21 ± 5.9	16 ± 2.7	0.132	0.180	0.041
p, p'-DDD[d]	23 ± 8.7	13 ± 19	0.18	15 ± 3.9	9.7 ± 1.6	0.026	0.180	1.000
dieldrin[b]	23 ± 6.8	8.1 ± 9.0	0.015	25 ± 5.9	14 ± 1.8	0.004	0.394	0.818

[a]see chapter 1 for complete list of analyte. [b] significant difference between sites, both years.
[c] significant difference between sites, 2002 only. [d] significant difference between sites, 2003 only.

p, p'-DDT and its metabolites. The bioavailable Σ DDT (sum of p, p'-DDT, p, p'-DDD and p, p'-DDE) concentrations were vary low at all sites in 2002 and 2003 (Table 4.2). Bioavailable ΣDDT concentrations were approximately 10-fold below the national and the Oregon fresh water aquatic life criteria 0.001 µg/L (*21, 22*) which is the level generally thought toxic to fish. The differences in bioavailable ΣDDT between Newberg and Corvallis sites in 2002 (p=0.18) and in 2003 (p=0.065) were not significant. In 2002, the bioavailable DDT profile was dominated by p, p'-DDD, followed by p, p'-DDE and p, p'-DDT (Figure 4.2). In 2003, bioavailable p, p'-DDE dominated, followed by p, p'-DDD and p, p'-DDT. This would be expected from older deposits and environmental degradation of p, p'-DDT to its metabolites. Bioavailable p, p'-DDD and p, p'-DDE concentrations detected at Newberg sampling sites in 2002 and 2003 were not significantly different (p=0.18) (Table 4.1). Bioavailable p, p'-DDE concentrations but not bioavailable p, p'-DDD concentrations at Corvallis sampling sites increased significantly between 2002 and 2003 (p=0.041 for p, p'-DDE and p=1 for p, p'-DDD). The mean concentrations of bioavailable p, p'-DDT at Newberg did not increase significantly between 2002 and 2003 (p=1), but the mean concentrations of bioavailable p, p'-DDT detected at Corvallis increased significantly (p=0.004). The cause of the increased p, p'-DDT concentration was not clear, but the results suggest possible new inputs or sediment disruption remobilizing DDT. However, the concentration differences observed were very small, so conclusions should be drawn prudently.

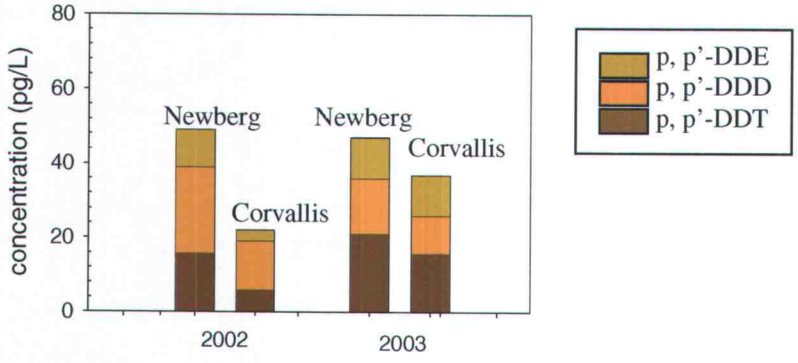

Figure 4.2 The mean concentration distribution of bioavailable p, p'-DDT, p, p'-DDD and p, p'-DDT at Newberg and Corvallis in 2002 and 2003

Dieldrin. Bioavailable dieldrin concentrations were significantly higher at Newberg sampling sites as compared to Corvallis sampling sites (p=0.015 for 2002 and p=0.004 for 2003) (Table 4.1). However, the bioavailable concentrations of dieldrin were very low and there was no significant difference between years. The bioavailable dieldrin concentrations detected were approximately 2000-fold below the national and the Oregon fresh water aquatic life criteria, 0.056 μg/L and were well below those generally thought to be toxic to fish (*23*). We are not aware of any reports linking picogram-per-liter concentrations of dieldrin to skeletal deformities.

To draw causation of fish deformities at Newberg due to chemical stresses, we can eliminate compounds that are absent, or if present, their concentrations do not differ from the Corvallis sampling sites where fish deformities are relatively low. The results indicated the difference between the presence or absence of bioavailable PCB congeners, p,p'- DDT and its metabolites, and dieldrin detected at the Newberg and Corvallis sampling sites was insignificant. For the compounds that were present there appeared to be little difference in their water concentrations between the sites. The results did not provide compelling support for the hypothesis that PCBs, DDTs and dieldrin present in surface water were a likely cause of the different deformity loads observed at Newberg versus Corvallis. The results coincided with the concentrations of these compounds found in fish ovarian/oocyte and sediment samples (*4*). Most organochlorine compounds were below detection limits in fish ovarian/oocyte and sediment samples and those that were detected were not significantly different between sites. In contrast, the results from the other collaborative group found metacercariae of a digenean trematode were directly associated with a large percentage of deformities detected in Willamette River fish and similar deformities were reproduced in laboratory fathead minnows exposed to cercariae extracted from Willamette River snail, see detail in Appendix E. Therefore, the weight of evidence suggested that parasitic infection, not chemical contaminants, was the primary cause of skeleton deformities observed in Willamette River fish.

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CHAPTER 5

CONCLUSIONS

As part of an assessment of the bioavailability of a chemical contaminant, this dissertation demonstrated the seasonal distribution of bioavailable organochlorine contaminants in a river system and evaluated the potential environmental factors influencing their bioavailability. The study was carried at the lower Willamette River at Portland Harbor, Oregon where surface water runoff is determined by climate influence and varies according to season. Bioavailable water concentrations of PCBs, DDTs, and dieldrin were determined using polyethylene membrane containing passive sampling device, known as semipermeable membrane device (SPMD). Our findings indicated that the influence of river seasonality on the bioavailable distributions of organochlorine contaminants was compound- and site-specific.

Seasonal variation of bioavailable ΣDDTs was site-specific. A seasonally significant increase of bioavailable ΣDDT concentrations was observed during low flow in summer at the sampling site just downstream of the former DDT production and handling facilities. The large bioavailable concentrations of ΣDDTs coincided with large DDD/DDE ratios. The ratio of DDD/DDE was highly variable, 6.6 ± 2.1 in summer, and 2.8 ± 1.3 in fall. A significant increase of bioavailable p, p'-DDD during low flow in summer was a major driver of seasonality in bioavailable ΣDDT concentrations. The linear regression analyses suggested seasonal changes in bioavailable p, p'-DDD concentrations were potentially related to changes in water temperature, river flow, and precipitation. Slow moving surface water, declining redox condition, elevated water temperature and decreased oxygen concentration during summer as observed in the present study would enhance anaerobic reductive dechlorination of p, p'-DDT to p, p'-DDD by both microbial degradation and chemical reaction in the sediment. In addition, the effect of increasing temperature in summer could decrease the organic matter-water partition coefficient of contaminants, resulting in

increasing bioavailable ΣDDTs release from the contaminated sediment. Evaluated together, increasing formation and release of p, p'-DDD in sediment during low flow in summer would contribute to the large increase of bioavailable p, p'-DDD and large ratio of DDD/DDE in surface water. As water temperature decreased and river flow increased in fall, the condition suitable for reductive dechlorination may have been altered, resulting in decreased bioavailable p, p'-DDD concentrations and change in bioavailable DDD/DDE ratios.

The above discussion contains speculation of the processes that highlights the lack of understanding on how seasonal river conditions interact to yield the observed seasonal variation in bioavailable p, p'-DDD and its relationship to bioavailable p, p'-DDE. Further research is needed such as confirmation of microbial communities responsible for reductive dechlorination of p, p'-DDT to p, p'-DDD or reductive sediment condition and their role in seasonal bioavailable p, p'-DDD distribution. Understanding the interaction of seasonal physico-chemical and biological factors will afford confidence in using this knowledge to predict the long-term seasonal effects of ΣDDTs on aquatic community and increased confidence in extrapolating information to other contaminated sites.

Bioavailable ΣPCB concentrations were found to have strong spatial and seasonal variation. Elevated concentrations of bioavailable ΣPCBs usually reached a maximum at the sampling sites within the industrial area. Both concentrations and daily loads of bioavailable ΣPCBs were significantly increased during high flow in fall, especially episodic rainstorms and the discernable seasonal pattern was observed at almost every sampling site along the 18-mile stretch of the study area. The results suggested the sources of bioavailable PCBs in surface water at the lower Willamette River were presumably seasonally dependent. Storm flow was especially important. Our results suggested storm events may have disturbed the interaction between PCBs and their associated organic matter resulting in remobilization of bioavailable fraction from sediments or particulate matter. In addition, the increase in bioavailable PCB concentrations and daily loads which was coincident with high precipitation and sewer overflows in fall suggested a significant contribution of PCBs from precipitation input, urban storm water and urban community wastewater discharges to the surface water.

The hypothesis that combined sewer overflows and precipitation could be a major source of PCBs during high precipitation and high river flow in the lower Willamette River warrants further investigation such as PCB fingerprinting to fully elucidate their contribution to PCB contamination. Further studies will be necessary to estimate the impact of the internal sources (i.e., contaminated sediments) and other sources (i.e., precipitation, runoffs, wastewater discharges) of PCBs and how much they contribute to the PCB problem in the system in order to reduce the PCB load and risk to the aquatic organism community.

Although no discernable spatial pattern was observed for dieldrin, an increase in bioavailable dieldrin concentrations and daily loads was observed at most sampling sites during high flow/high precipitation in fall. The seasonal pattern of bioavailable dieldrin distribution illustrated the transport behavior for non-point source contaminants which were widely dispersed and had no intentional seasonal application cycle such as with the current use pesticides. Our results combined with the historical use of dieldrin in the watershed suggested bioavailable dieldrin transport from contaminated site upstream including erosional inputs to the river during precipitation runoff from land sources or riverbed sediment.

To screen the potential impacts associated with seasonal changes of bioavailable organochlorines on aquatic organisms, the bioavailable concentrations of ΣDDTs, ΣPCBs and dieldrin were compared to the national recommended water quality criteria and the Oregon water quality criteria. Seasonal exceedances of the criteria for ΣDDTs were limited to only the sampling site downstream of the former DDT production and handling facilities during low flow in summer. Exceedances of the criteria for dieldrin were frequently observed during high flow in fall. In contrast, no discernable seasonal pattern of criteria exceedance was observed for ΣPCBs. These findings suggested the potential impacts associated with seasonal changes of bioavailable organochlorine distributions in surface waters and the significances of considering seasonal, chemical-, and site-specific conditions in risk assessment and water quality management.

Importantly, predicting bioconcentration from measured water concentrations of bioavailable organic contaminants using passive sampling devices can provide more accurate bioconcentration factor (BCF) compared to using conventional filtered water methodology. Measured or predicted BCFs are an essential component of both human and environmental risk assessment because BCFs illustrate the importance linkage between the water column and its influence on exposure of aquatic organisms. BCFs are strongly influence prediction of toxic effects, especially when chemical residue-based dose-response relationships are used (Barron, 1990 and Cook and Burkhard, 1996). Many hydrophobic organic contaminants such as PCBs and organochlorine pesticides have a high affinity for particulates and dissolved organic matter, which can reduce bioavailability of chemicals. Predicting BCFs from dissolved water concentrations using conventional filtered water methodology would include contaminants associated with dissolved organic matter, which could not transport across biomembranes. Thus, it will underestimate the BCF. In contrast, predicting BCFs using passive sampling devices (BCF_{PSD}) accounts for the bioavailability consideration and consequently reduce uncertainty for site-specific bioavailability conditions.

The present study assesses the sampling device attributes. For instance, the passive sampling technique can be successfully used to assess spatial and seasonal distribution of bioavailable organochlorines in surface water, as well as, to allow a direct assessment of the water quality based on bioavailable concentrations, as presented in Chapter 2. Also, it can be used as a tool to assess the potential for direct exposure to hydrophobic organic contaminants and to determine whether these contaminants are likely cause(s) of the adverse effect(s) observed in aquatic organisms, as presented in Chapter 4 and Appendix E. However, an inexpensive and simpler time-integrative passive sampling device is still needed.

In response to a growing need of an alternative passive sampling device, our research group has further developed a triolein-free low density polyethylene membrane lay flat tubing (LFT). Our laboratory and field verification demonstrated that the LFT without triolein proved reliable and had the same benefits as SPMD, but was simpler, inexpensive and lacked the interference from triolein impurities as found in SPMD. Our results found that LFT tended to accumulate compounds with high log K_{ow} faster than SPMD. Also, buildup of biofouling on the device exterior surface and approaching equilibrium during field exposure

appeared not to be a concern as compared to SPMD. LFT sampling rates were estimated and modeled for 33 target analytes, including PAHs, PCBs, and organochlorine pesticides. The successful determination of field derived data illustrated the effectiveness and reliability of LFT for environmental monitoring. Its application increases the range of tools available to those involved in monitoring water quality. The method of choice for environmental monitoring will depend on the contaminant nature and environmentally relevant concentrations of contaminants as well as the objective of a study.

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Appendix A. Estimated river concentrations of bioavailable p, p'-DDT and its metabolites, dieldrin, and Σ PCBs (sum of 25 congeners) at the Willamette River during summer and fall of 2001 to 2004. River concentrations were estimated from SPMD concentrations. Deployments of SPMDs were typically 7- to 21-day long.

sampling event	p,p'-DDE (pg/L)	p,p'-DDD (pg/L)	p,p'-DDT (pg/L)	Σ DDTs (pg/L)	dieldrin (pg/L)	Σ PCBs (pg/L)
detection limit (pg/L) [a]	2-15	3-7	3-7		3-19	0.69 – 54[b]
August 2001						
RM 1[c]	66 (5.3)	167 (49)	2.3 (3.3)	236 (51)	43 (2.0)	13 (11)
RM 3.5 West	44	126	4	174	47	14
RM 3.5 East	55	250	5	309	58	31
RM 7 West	125	514	48	687	57	31
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	48	112	bdl	161	75	13
RM 12	15	59	bdl	74	42	14
RM 13	18	56	5	79	41	18
RM 15	11	20	bdl	31	23	4.8
RM 17	13	46	13	72	37	8.5
RM 18[c]	11 (4.1)	42 (25)	7.5 (11)	60 (40)	35 (15)	10 (8.2)
September 2001						
RM 1[c]	120 (1.5)	289 (32)	6.0 (8.5)	414 (39)	65 (2.5)	120 (13)
RM 3.5 West	66	327	bdl	393	50	89
RM 3.5 East	68	371	bdl	439	55	94
RM 7 West	201	956	100	1257	108	63
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	114	481	bdl	595	71	123
RM 12	22	112	bdl	134	34	12
RM 13	28	73	14	115	50	13
RM 15	30	144	11	184	38	4.2
RM 17	33	61	18	111	63	17
RM 18[c]	21 (0.77)	57 (68)	4.6 (6.5)	82 (60)	45 (3.0)	13 (4.8)
October 2001						
RM 1[c]	82 (10)	125 (18)	32 (3.7)	239 (25)	56 (2.7)	130 (0.35)
RM 3.5 West	NA	NA	NA	NA	NA	NA
RM 3.5 East	NA	NA	NA	NA	NA	NA
RM 7 West	87	266	168	521	59	72
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	33	35	14	83	34	36
RM 12	21	25	18	64	40	29
RM 13	27	34	18	79	40	26
RM 15	23	13	10	45	31	16
RM 17	18	20	13	51	26	21
RM 18[c]	26 (0.32)	28 (2.8)	16 (0.12)	69 (2.4)	62 (0.44)	35 (1.1)
November 2001						
RM 1	71	73	26	170	75	104
RM 3.5 West	NA	NA	NA	NA	NA	NA
RM 3.5 East	114	140	76	330	137	270
RM 7 West	93	180	76	349	81	94
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	50	33	22	104	78	101
RM 12	46	27	32	105	83	49
RM 13	67	42	26	135	101	100
RM 15	56	29	21	106	102	37
RM 17	50	30	18	98	104	105
RM 18	NA	NA	NA	NA	NA	NA
July 2002						
RM 1[c]	44 (11)	95 (6.3)	7.1 (0.72)	146 (18)	24 (1.2)	11 (1.7)
RM 3.5 West	54	159	13	226	54	33

sampling event	p,p'-DDE (pg/L)	p,p'-DDD (pg/L)	p,p'-DDT (pg/L)	Σ DDTs (pg/L)	dieldrin (pg/L)	Σ PCBs (pg/L)
detection limit (pg/L) [a]	2-15	3-7	3-7		3-19	0.69 – 54[b]
RM 3.5 East	52	147	14	212	53	52
RM 7 West	160	939	143	1242	71	47
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	43	70	13	126	65	35
RM 12	25	42	9	76	51	13
RM 13	45	68	8	121	74	32
RM 15	24	30	8	63	38	6.4
RM 17	29	31	7	68	50	8.5
RM 18[c]	18 (4.1)	24 (6.5)	4.3 (1.3)	47 (12)	44 (15)	8.1 (2.7)
August 2002						
RM 1	52 (2.5)[c]	85 (2.6)[c]	17 (1.8)[c]	154 (3.4)[c]	28 (0.97)[c]	15
RM 3.5 West	61	218	20	300	62	45
RM 3.5 East	66	229	24	319	66	60
RM 7 West	129	1207	130	1465	67	52
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	37	63	12	112	48	37
RM 12	23	33	10	66	41	19
RM 13	25	41	11	76	47	26
RM 15	23	33	9	65	46	9.3
RM 17	23	31	13	67	60	11
RM 18[c]	18 (4.1)	25 (5.3)	10 (2.4)	53 (12)	41 (7.3)	12 (2.6)
September 2002						
RM 1[c]	49 (2.5)	96 (18)	11 (1.8)	156 (22)	32 (5.6)	26 (2.2)
RM 3.5 West	NA	NA	NA	NA	NA	NA
RM 3.5 East	52	175	18	245	43	56
RM 7 West	90	759	74	923	39	52
RM 7 East[d]	37 (10)	68 (21)	20 (12)	126 (39)	27 (10)	55 (42)
RM 8	27	39	7	74	28	24
RM 12	22	25	11	58	28	12
RM 13	29	38	11	78	30	22
RM 15	21	22	7	49	23	3.0
RM 17	15	16	8	39	22	bdl
RM 18[c]	18 (0.61)	22 (1.3)	9.8 (1.1)	50 (1.7)	33 (0.14)	9.5 (1.6)
November 2002						
RM 1[c]	91 (3.6)	102 (11)	11 (1.2)	204 (16)	83 (8.0)	29 (3.5)
RM 3.5 West	78	108	14	200	69	32
RM 3.5 East	61	79	14	153	65	37
RM 7 West	129	321	55	505	64	30
RM 7 East[c]	43 (4.8)	41 (5.4)	7.6 (0.079)	92 (10)	50 (0.29)	16 (3.7)
RM 8	37	28	7	73	46	10
RM 12	38	24	8	70	46	5.6
RM 13	45	35	9	88	53	29
RM 15	47	27	9	83	53	4.9
RM 17	13	7	6	27	13	bdl
RM 18[c]	52 (8.0)	37 (9.2)	10 (1.1)	99 (18)	84 (24)	51 (7.3)
October 2003						
RM 1[c]	76 (12)	170 (4.5)	14 (1.5)	260 (18)	47 (2.6)	48 (2.6)
RM 3.5 West	54	242	36	332	54	69
RM 3.5 East	59	242	37	338	61	102
RM 7 West	105	426	149	680	48	61
RM 7 East[e]	47 (8.9)	51 (12)	8.9 (6.9)	107 (25)	40 (22)	41 (14)

sampling event	p,p'-DDE (pg/L)	p,p'-DDD (pg/L)	p,p'-DDT (pg/L)	Σ DDTs (pg/L)	dieldrin (pg/L)	Σ PCBs (pg/L)
detection limit (pg/L) [a]	2-15	3-7	3-7		3-19	0.69 – 54[b]
RM 8	24	34	6	65	34	35
RM 12	NA	NA	NA	NA	NA	NA
RM 13	NA	NA	NA	NA	NA	NA
RM 15.5	23	26	bdl	49	35	1.5
RM 17	13	17	bdl	30	24	bdl
RM 18.5	14	15	bdl	29	29	bdl
November 2003						
RM 1[c]	264 (17)	223 (8.1)	39 (9.6)	527 (35)	76 (5.9)	39 (3.5)
RM 3.5 West	NA	NA	NA	NA	NA	NA
RM 3.5 East	166	119	37	322	86	80
RM 7 West	199	232	102	533	76	22
RM 7 East [c]	67 (6.7)	50 (6.0)	14 (0.53)	131 (13)	67 (2.8)	15 (6.7)
RM 8	70	44	13	127	64	13
RM 12	58	26	16	101	60	bdl
RM 13	81	38	20	139	68	5.4
RM 15	69	32	17	118	74	4.3
RM 17	53	24	16	93	58	bdl
RM 18	54	21	16	91	62	5.5
July 2004						
RM 1[c]	50 (1.9)	102 (7.4)	9.0 (0.18)	161 (9.5)	35 (2.7)	90 (6.3)
RM 3.5 West	57	234	21	311	55	128
RM 3.5 East	42	133	14	188	47	154
RM 7 West	111	804	132	1046	52	126
RM 7 East	29	66	8	103	39	99
RM 8	26	54	8	88	45	90
RM 12	28	39	bdl	67	50	89
RM 13	28	39	7	75	45	90
RM 15	23	28	6	58	43	42
RM 17	34	43	9	86	64	34
RM 18[c]	19 (4.4)	22 (2.9)	6.0 (1.3)	47 (8.5)	38 (7.6)	42 (3.3)
August 2004						
RM 1[c]	91 (0.52)	126 (2.1)	56 (1.1)	273 (2.7)	68 (2.8)	95 (2.9)
RM 3.5 West	63	149	25	236	62	152
RM 3.5 East	52	120	29	201	65	123
RM 7 West	106	546	161	813	69	116
RM 7 East	NA	NA	NA	NA	NA	NA
RM 8	30	50	11	91	67	104
RM 12	25	36	13	74	68	69
RM 13	28	37	18	83	60	92
RM 15	31	43	17	90	68	51
RM 17	19	20	8	48	51	35
RM 18[c]	23 (0.23)	29 (1.0)	15 (5.2)	67 (4.0)	60 (2.0)	53 (4.6)
October 2004						
RM 1[c]	122 (1.1)	139 (3.0)	28 (0.27)	290 (4.4)	108 (1.5)	260 (3.1)
RM 3.5 West	70	104	29	203	71	152
RM 3.5 East	82	98	29	209	93	408
RM 7 West	282	656	403	1341	105	184
RM 7 East	31	30	8	69	66	63
RM 8	45	41	10	96	85	95
RM 12	32	28	10	71	69	64
RM 13	56	53	21	129	84	162

sampling event	p,p'-DDE (pg/L)	p,p'-DDD (pg/L)	p,p'-DDT (pg/L)	Σ DDTs (pg/L)	dieldrin (pg/L)	Σ PCBs (pg/L)
detection limit (pg/L) [a]	2-15	3-7	3-7		3-19	0.69 – 54[b]
RM 15	49	39	10	98	86	46
RM 17	37	27	9	73	86	49
RM 18 [c]	58 (0.58)	47 (0.93)	18 (1.2)	124 (2.7)	123 (0.90)	68 (3.2)

n = 1 composite sample of 5 SPMDs; duplicate samples were at River Mile 1 and 18; values in parenthesis are 1SD; bdl- below detection limit; NA-data not available

[a] range of method detection limits from 13 sampling events

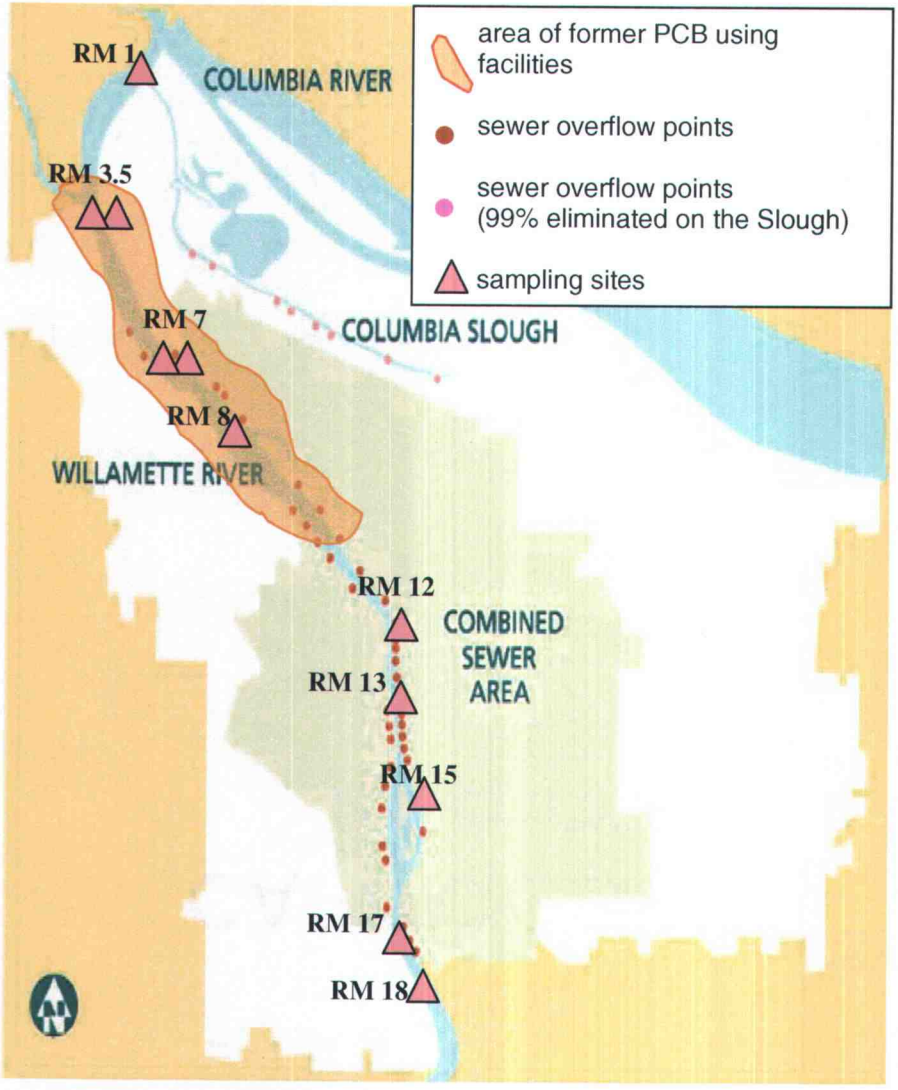
[b] detection limits for 25 individual PCB congeners

[c] n = 2

[d] n = 19

[d] n = 15

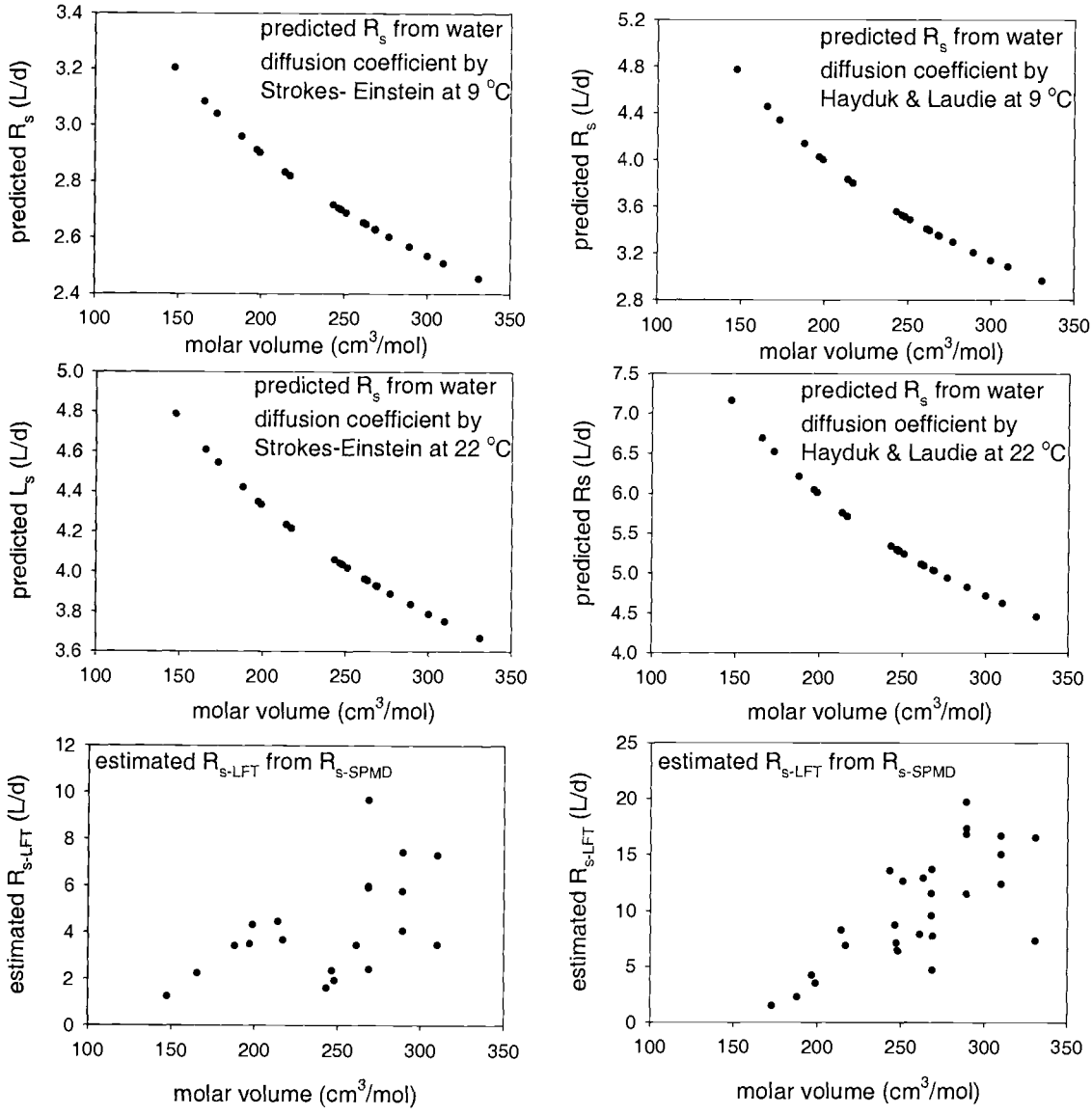
Appendix B. Sewer overflow points in Portland area (City of Portland, 2004, http://www.portlandonline.com/bes/index.cfm?c=29323)



Appendix C. Equations used to estimate molecular diffusitivity in water ($D_{i\,w}$)

Stokes-Einstein relation	$D_{iw} = \frac{kT}{6\pi\eta r_i}$	where k = the Boltzmann constant $(1.381 \times 10^{-23}$ kg m^2 s^{-2} K$^{-1})$ η = dynamic viscosity (kg m^{-1} s^{-1}) T = temperature (K) r_i = molecular radius $= [3V_i/4\pi N_A]^{1/3}$ where V_i = molar volume of the chemical (cm^3 mol^{-1}) N_A = Avogadro's number $(6.02 \times 10^{23}$ mol$^{-1})$
Hayduk and Laudie	$D_{iw} = \frac{13.26 \times 10^{-5}}{\eta^{1.14} V_i^{0.589}}$	where η = solution viscosity (10^{-2} g cm^{-1} s^{-1}) at the temperature of interest V_i = molar volume of the chemical (cm^3 mol^{-1})

Appendix D. Predicted values for LFT sampling rates using different methods.



Appendix E. *"Environmental stresses and skeletal deformities in fish from the Willamette River, Oregon".*

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Environmental Stresses and Skeletal Deformities in Fish from the Willamette River, Oregon

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The Willamette River, one of 14 American Heritage Rivers, flows through the most densely populated and agriculturally productive region of Oregon. Previous biological monitoring of the Willamette River detected elevated frequencies of skeletal deformities in fish from certain areas of the lower (Newberg pool [NP], rivermile [RM] 26−55) and middle (Wheatland Ferry [WF], RM 72−74) river, relative to those in the upper river (Corvallis [CV], RM 125−138). The objective of this study was to determine the likely cause of these skeletal deformities. In 2002 and 2003, deformity loads in Willamette River fishes were 2−3 times greater at the NP and WF locations than at the CV location. There were some differences in water quality parameters between the NP and CV sites, but they did not readily explain the difference in deformity loads. Concentrations of bioavailable metals were below detection limits (0.6−1 μg/L). Concentrations of bioavailable polychlorinated biphenyls (PCBs) and chlorinated pesticides were generally below 0.25 ng/L. Concentrations of bioavailable polycyclic aromatic hydrocarbons were generally less than 5 ng/L. Concentrations of most persistent organic pollutants were below detection limits in ovary/oocyte tissue samples and sediments, and those that were detected were not significantly different among sites. Bioassay of Willamette River water extracts provided no evidence that unidentified compounds or the complex mixture of compounds present in the extracts could induce skeletal deformities in cyprinid fish. However, metacercariae of a digenean trematode were directly associated with a large percentage of deformities detected in two Willamette River fishes, and similar deformities were reproduced in laboratory fathead minnows exposed to cercariae extracted from Willamette River snails. Thus, the weight of evidence suggests that parasitic infection, not chemical contaminants, was the primary cause of skeletal deformities observed in Willamette River fish.

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Introduction

The Willamette River in western Oregon is one of 14 American Heritage Rivers and receives more runoff per square mile watershed than any other river in the U.S (*1*). It flows north for ~187 miles through mixed agricultural and urban areas to Portland, Oregon's largest metropolitan area, before joining the Columbia River (Figure 1). The Willamette basin is home to 70% of Oregonians, and the Willamette Valley is one of the most highly productive agricultural regions in the Pacific Northwest (*2*−*3*). The Willamette River is a significant migratory corridor, nursery, and spawning habitat for salmon, and nearly 50 species of fish have been identified in the river (*3*). Recreational fishing is popular, and resident species are fished throughout the year.

In the early 1990s, the Oregon Department of Environmental Quality initiated investigations of skeletal deformities in Willamette River fishes. Biological monitoring has been widely used to evaluate aquatic ecosystem health and potential impacts of anthropogenic activities. It has been suggested that skeletal deformities in fish serve as a useful bioindicator of pollution (*4*−*6*), and evaluation of skeletal deformities in juvenile fish has been used to monitor the health of fish populations (*7*−*11*). In 1992−1994, the incidence of skeletal deformities in northern pikeminnow (*Ptychocheilus oregonensis*) collected from the Newberg (NP) region, extending from river mile (RM) 55 to 26.5 (Figure 1), ranged from 22 to 74% (*12*−*13*). Northern pikeminnow skeletal deformity rates were also elevated (21.7%) in the middle Willamette River (around RM 72, Wheatland Ferry; Figure 1). In contrast, the skeletal deformity rates in juvenile northern pikeminnow collected from the upper Willamette River (RM 185−125) ranged from 1.6 to 5.3% (*12*−*13*). Northern pikeminnow was not the only species impacted. Of 15 species collected from the Newberg region and associated tributaries in 2000, skeletal deformity rates exceeded 25% in 10 species (*14*). As a whole, biomonitoring of skeletal deformities in Willamette river fish suggested that fish from the Newberg region and middle Willamette River had significantly greater deformity rates than fish from the upper Willamette River.

In the mid-late 1990s, proposals to tap the Newberg region of the Willamette River as a source of drinking water for urban expansion heightened public concern related to the reports of deformed fish (http://www.hevanet.com/safewater/recentnewshome.htm). In 1998, for example, 85% of people surveyed expressed "extreme" concern about the level of toxic chemicals in the river (Oregon Daily Emerald, Feb. 26, 1998). This research was a response to such concerns and, especially, scientific uncertainty concerning potential causes.

A wide variety of chemical, physical, and biological stressors have been associated with skeletal deformities in fish. A variety of chemicals, including heavy metals, such as lead and numerous organophosphate pesticides, are known to induce neuromuscular damage that can result in skeletal deformities (*15*−*18*). Chemicals can also cause skeletal deformities by impairing developmental processes and bone formation. Compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polychlorinated biphenyls, toxaphene, and cadmium have been reported to cause skeletal deformities

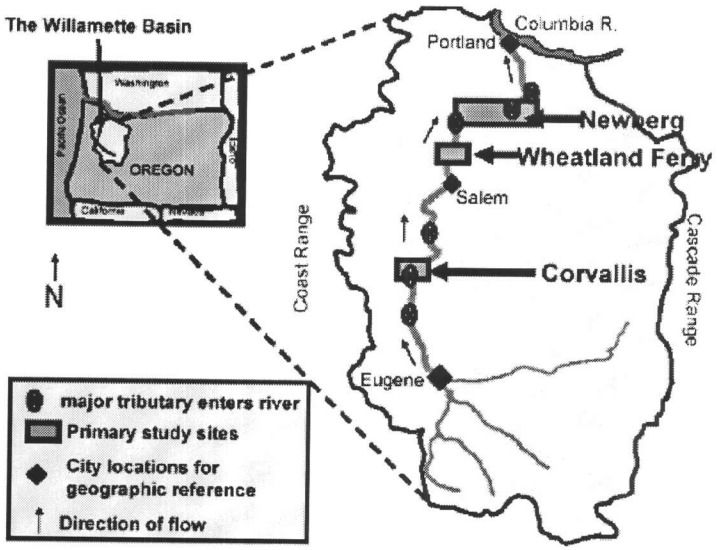

FIGURE 1. Diagram of the Willamette Basin depicting the general location and course of the Willamette River and primary study locations.

through such mechanisms (*19*–*24*). Skeletal deformities have also been linked to water quality problems, including low pH (*25*–*26*), low dissolved oxygen (*27*–*28*), and elevated temperatures (*29*–*30*). Nutritional deficits, particularly ascorbic acid and tryptophan deficiencies, have been linked to skeletal deformities in fish (*31*–*32*). Inbreeding has also been shown to cause skeletal deformities, including scoliosis, lordosis, curved neural spines, fused vertebrae, and compressed vertebrae (*33*–*35*). Finally, numerous infectious biological agents, including viruses, bacteria, and parasites, have been reported to cause skeletal deformities (*36*–*39*). Although the association of fish skeletal deformities with a wide variety of stressors makes it a useful endpoint for biological monitoring, the observation of a high incidence of skeletal deformities, alone, has little diagnostic value.

The purpose of this study was to identify or diagnose the cause(s) of skeletal deformities associated with Willamette River fish, with particular emphasis on the Newberg region. Skeletal deformities in fish collected from the upper, middle, and lower Willamette River in 2002 and 2003 were characterized to determine whether recent prevalences were similar to those reported previously and to further describe spatial and temporal patterns. In situ monitoring of river water quality coupled with in situ sampling and analysis of bioavailable organic contaminants and metals was used to compare water quality and potential for direct exposure to known chemical contaminants at the Newberg and Corvallis study sites and determine whether these factors were likely causes of the deformities. Analysis and comparison of sediment samples and fish tissue from Newberg and Corvallis was used to evaluate potential trophic or maternal transfer of known persistent organic pollutants (POPs) as a potential cause. Bioassay of river water extracts using embryo–larval fathead minnows (*Pimephales promelas*) exposed under controlled laboratory conditions was used to evaluate the potential role of unknown chemicals or complex chemical mixtures in causing the skeletal deformities observed in Willamette River fish. Field-collected fish were examined for parasites, and the association of parasitic infection with skeletal deformities was quantified. Finally, cercariae of a trematode parasite (identified as *Apophallus donicus*) were collected from Willamette River snails (*Fluminicola virens*, the intermediate host for *A. donicus*), and fathead minnows were exposed to the cercariae in the laboratory. Together, these components provided a weight-of-evidence-based, empirical approach to identify the likely cause of skeletal deformities in Willamette River fishes.

Methods

Fish Collection and Deformity Characterization. Larval and juvenile fish were sampled from May to October 2002 and May to August 2003. Fish were collected by beach seine, cast net, and dip net. The three primary sampling areas were Newberg pool (NP; RM 47.5–53; lower Willamette), Wheatland Ferry (WF; RM 72–74; middle Willamette), and Corvallis (RM 125–138; upper Willamette). Specimens were euthanized with an overdose of MS-222 (Finquel (tricaine methanesulfonate) Argent Chemical Laboratories, Redmond, WA at 500 ppm) and fixed in 10% buffered formalin. Seventeen species were collected, with *Prychocheilus oregonensis* (northern pikeminnow), *Richardsonius balteatus* (redside shiner), *Catostomus macrocheilus* (largescale sucker), *Mylocheilus caurinus* (peamouth), and *Acrocheilus alutaceus* (chiselmouth) representing the most commonly collected species (total sample sizes > 1000) (*40*).

Specimens fixed for a minimum of two weeks were X-rayed in a Faxitron MX-20 cabinet X-ray machine using AGFA Structurix D4 DW ETE industrial radiography film. Film was developed using a Kodak X-OMAT model M6B developer. Radiographs of ~15 700 fish were inspected for deformities using a 10–15× ocular over a light table. Presence or absence of 12 different categories of skeletal deformities was scored (additional details in ref *40*), and our analyses were based on the number of deformity categories present per individual (deformity load) (*14*) plus the number of precaudal deformities, since caudal deformities are uniformly distributed (*40*). On the basis of random reevaluation of 550 fish, reader error was not significant (*40*).

In Situ Water Quality and Bioavailable Contaminants. *Site Description and Sample Collection.* Willamette River sampling sites were chosen to facilitate investigation of seasonal and spatial bioavailable contaminant concentrations at Newberg and Corvallis (Figure 1). Two sampling stations were located at Newberg, one on the south side of the river (RM 47; N 45°16.02′, W 122°54.59′) and one a few miles

downriver on the north side of the river (RM 44; N 45°15.27', W 122°53.58'). Two stations were located at Corvallis (RM 135; [1] N 45°29.13', W 122°39.06'; [2] N 45°27.37', W 122°39.47'). Newberg station 1 (RM 47) was about 25–30 ft from the shoreline, and the local water depth was 27 ft. Newberg station 2 (RM44) was about 30 ft from the shore, and the local water depth was 20 ft. Both Corvallis stations were about 15 ft from the shore, and the local water depth was 7–11 ft. Flow near the Corvallis sites in May 2002 was ~9000 to 10 000 ft^3/s and by late July had decreased to ~4500 ft^3/s. At the Newberg sites, flow in May 2002 was ~17 000 to 20 000 ft^3/s and by late July decreased to ~7000 ft^3/s. The Corvallis area was generally shallow (2–12 ft) and characterized by shallow gravel and sediment riprap. In the Newberg area, the Willamette River was much deeper (20–60 ft), and there were no shallow gravel beds near the study sites. The Willamette River has very steep banks at the Newberg area; within 5–10 ft of the bank, the river is 15–20 ft deep. The bottom of the river in this area is a combination of rock and mud.

Water sampling was conducted from May to July in 2002 and 2003. Three 21-d sampling events were completed per year, one each in approximately May, June, and July. Sampling events were designed to characterize river conditions during spawning and early development. Nutrient and water quality parameters, including dissolved oxygen, specific conductance, salinity, total dissolved solids, temperature, pH, ORP (oxidation reduction potential), depth, ammonium, nitrate, and turbidity, were collected on an hourly basis with a YSI 6920 Sonde (YSI, Yellow Springs, OH).

Dissolved, bioavailable organic contaminants and metals were collected by deploying passive sampling devices (PSD) and diffusive gradient thinfilms (DGT) in protective mesh cages. PSDs consisted of neutral lipid (i.e., triolein) enclosed in layflat polymeric tubing (Environmental Sampling Technologies, St. Joseph, MO) (*41*). Five individual PSDs and one DGT (DGT Research Ltd, UK) were included in each cage. Each cage was suspended with a "float-cable-cage-cable-anchor" arrangement that ensured that the cage would stay at the station and would stay suspended one foot from the river bottom. The five PSDs were later composited for analysis. PSDs and DGTs were kept on ice in sealed, airtight, amber glass containers during transport to and from the field sites. Complete PSD descriptions have been published (*42*). PSDs were gently cleaned of sediment or algae after deployment at the site utilizing a tub filled with site water to minimize air exposure. No fouling impedance was employed in calculations of estimated water concentration, since algae growth on the devices was nil to minimal.

Analytical Procedure. PSDs were extracted by hexane dialyses, in amber glass jars. Sample volumes were reduced using a TurboVap LV (Zymark Corp, Hopkinton, MA). The samples were then run through gel permeation chromatograph (GPC) (models: 515 pump, 2487 dual wavelength absorbance detection, 717 auto-sampler, and fraction collector II, Waters, Corp. Milford MA), and fractions containing organochlorine pesticides, organophosphate pesticides, organonitrogen pesticides, PCBs, and PAHs were collected. The GPC columns were 19 mm × 300 mm divinylbenzene copolymer particles, 15-um particle size and 100-Å pore size. The GPC program ran with 100% dichloromethane at 5.0 mL/min. Appropriate fractions were determined by analyzing standards and fortified samples (*43*). Appropriate fractions were analyzed using GC-dual-ECD (organochlorines), GC-dual-NPD (organonitrogen and organophosphate pesticides), and HPLC–DAD and fluorescence (PAHs) (GC 6890N, Agilent Technologies, Pal Alto CA and HPLC 1100, Hewlett-Packard, Pal Alto CA). Sample manipulations were performed in either brown amber or foil-wrapped glass containers to minimize UV/vis exposure. Detailed analytical methods used for quantification of organochlorines, organonitrogen pesticides, and organophosphate pesticides are provided elsewhere (*43*).

The polycyclic aromatic hydrocarbons (PAH) contaminants fraction was separately concentrated to ~1.0 mL. PAH detection and quantitation was performed on a HPLC with dual detection by flourescence or diode array, both with multiple wavelengths. The fluorescence detector had an excitation wavelength at 230 and emission wavelengths at 360, 410, and 460 nm; the diode array had detection signals at 254, 242, and 230 nm. Only three compounds, fluorene, acenaphthylene, and indeno(123*cd*)pyrene, were detected by diode array; the rest were detected with the fluorescence detector. The column used was a Phenomenex Luna C18, with 3-μm particle size. The instrument was run with a constant flow rate of 0.75 mL/min and a timed gradient for the acetonitrile/water eluent system. The time program ran at 40% acetonitrile for 10 min, was gradually ramped up to 70% acetonitrile for 15 min, and then ramped up to 90% acetonitrile for 10 min. The program was held at 90% acetonitrile for 3 min and then returned to 40% and analyzed by HPLC with diode array detection (DAD) and fluorescence detection. The approximate retention times in min are naphthalene, 16.0; acenaphthylene, 16.9; fluorene, 18.1; phenanthrene, 18.15; anthracene, 18.6; fluoranthene, 19.0; pyrene, 20.0; chrysene, 20.4; benzo(*a*)anthracene, 22.1; benzo(*b*)fluoranthene, 24.5; benzo(*k*)fluoranthene, 24.8; benzo(*a*)pyrene, 25.1; dibenzo(*a,h*)anthracene, 27; benzo(*g,h,i*)-perylene, 28.8; and indo(1,23,*cd*)pyrene, 29.2.

After DGTs were retrieved and in the laboratory, the resin-gel was removed and immersed for 24 h in 1.0 mL of 1 M trace metal grade nitric acid (Fisher Scientific). Acetic acid and sodium acetate were used as the supporting electrolyte, and the samples were diluted to a final volume of 25 mL with 18-MΩ·cm water. The analysis was by anodic stripping voltammetry (ASV) (TraceDetect, Seattle, WA). All grab water samples were filtered thru a 0.45-μm membrane filter prior to metal analyses by ASV. ASV was used to quantify the metals reported. Reduction potentials were verified with standards for each metal tested.

Quality Control (QC). Field, trip, and extraction blanks were used with each sampling event. Field blank PSD samples were opened and exposed to the atmosphere during deployment or recovery. Field blanks were processed and analyzed exactly as deployed PSD samplers. Field extraction blanks were opened in the field and washed simulating the process of removing the light sediment or algae on the passive sampling devices. Samples containing residues exceeding the blanks were considered positive for residues. Transport blank values were multiplied by the water volume they would have been exposed to if left with the other PSDs. The Corvallis site was designated as a field duplicate site. Field duplicates represented 30% of all samples collected. All QC sample types were included in each analytical batch. Laboratory QC samples included reagent blanks, fortified samples, and laboratory duplicates. Each QC type represented 5–10% of the total number of samples analyzed in any given batch. They were prepared and analyzed in the same fashion as the field samples. Organic standard (ChemService, West Chester, PA) curves were typically composed of ≥4 standard concentrations and metal standard (Alfa Aesar, Ward Hill, MA) curves ≥3 for all analyses.

Data Analysis. The theory and mathematical models required for estimation of analyte water concentrations from the concentration in the PSD lipid have been described (*42*). The following equation was used to calculate the dissolved (bioavailable) water concentration,

$$C_w = C_{SPMD} M_{SPMD} / R_s t$$

where C_w is the concentration of analyte in water. C_{SPMD} is

the concentration in lipid (SPMD), t is the exposure time in days, M_{SPMD} is the mass of SPMD in g, and R_s is the PSD sampling rate. Sampling rates (R_s) for a large series of OC and PAH contaminants have been previously established.

The mass of the metal in the DGT resin gel (M) was determined from the ASV quantitation. The theory and mathematical models required for estimation of the analyte water concentrations from the concentration in the DGT have been previously described (*44*). The following equation was used to calculate the labile (bioavailable) water concentration,

$$M = C_e(V_{HNO_3} + V_{gel})/f_e$$

where C_e was the concentration of metals in the 1 M HNO_3 elution solution, V_{HNO_3} was the volume of HNO_3 added to the resin gel, V_{gel} was the volume of the resin gel, and f_e was the elution factor for each metal. The concentration of the metal measured by DGT (C_{DGT} = "bioavailable" water concentration) was determined from the following equation,

$$C_{DGT} = M\Delta g/(DtA)$$

where Δg was the thickness of the diffusive gel (0.8 mm) plus the thickness of the filter membrane (0.13 mm), D was the diffusion coefficient of metal in the gel, t was deployment time, and A was the exposure area (A = 3.14 cm^2) (*44*).

Analysis of POPs in Northern Pikeminnow Ovary Tissue. Northern pikeminnow (*P. oregonensis*) was the species chosen for analysis of maternal transfer of POPs. They are abundant at both study locations, relatively easy to collect, reach moderately large sizes, and have large number of deformities in the Newberg region (*12, 14*). Adults were collected from Newberg (N 45°16.007′, W 122°55.031′) and Corvallis (N 44°28.250′, W 123°14.300′) (Figure 1) in May−June 2002 using a combination of hook and line and electrofishing and transported to the laboratory on ice. Wet weights ranged from 375 to 975 g, and there was no significant difference in the mean wet weight of the fish collected from the two study sites. Ovarian tissue and associated oocytes were removed from gravid females using clean, solvent-rinsed, dissection tools; placed into certified I-Chem jars; and stored at −20 °C until extracted.

Samples were shipped to GLP (Good Laboratory Practices)-certified analytical laboratories for quantification of a range of POPs. Twenty-one chlorinated pesticides were quantified by gas chromatography with electron capture detection (GC/ECD) according to EPA method 8081A (ODEQ laboratory, Portland OR). Twenty-eight polychlorinated biphenyl (PCB) congeners were quantified by GC/ECD according to EPA method 8082 (ODEQ laboratory, Portland, OR). Additionally, concentrations of seven polychlorinated dibenzo-*p*-dioxin (PCDD) congeners and 10 polychlorinated dibenzofurans (PCDFs) were quantified by high-resolution GC/MS (Axys Analytical, British Columbia, Canada). Method detection limits (MDLs) for chlorinated pesticides and PCBs ranged from 2.5 to 3.3 μg/Kg wet wt. MDLs for PCDDs and PCDFs ranged from 0.1 to 0.13 ng/Kg wet wt.

Five ovarian tissue/oocyte samples (each from a separate fish) were analyzed per study area. For statistical analysis and plotting of figures, concentrations below the method reporting limit (MRL) or detection limit were assumed to be equal to one-half of the limit. When assumptions of parametric statistics were met, *t*-tests were used to test for differences among study sites. Kolmogorov−Smirnov's test was used in cases that parametric assumptions were not met.

Analysis of POPs in Sediment. Grab samples of surficial sediment were collected from Newberg and Corvallis sites. In 2002, three samples were collected at Newberg location N 45°16.308′, W 122°59.460′, and three samples were collected at Corvallis location N 44°31.567′, W 127°15.384′. In 2003, three samples were collected at Newberg location N 45°15.567′, W 122°54.231′, and three samples were collected at Corvallis location N 44°32.887′, W 123°15.432′. Sediment samples were scooped directly into certified I-Chem jars, transported on ice to the laboratory, and stored at −20 °C until shipped for analysis. Sediment samples were extracted and analyzed for 22 chlorinated pesticides by GC/ECD (EPA method 8081A), 8 nitrogen/phosphorus pesticides by GC/NPD, and 29 PCB congeners by GC/ECD (EPA method 8082 A) at the ODEQ laboratory, Portland, OR. MDLs for chlorinated pesticides and PCBs were ~0.33 μg/Kg wet wt. The MDL for nitrogen/phosphorus pesticides was 10 μg/Kg wet wt.

Skeletal Deformities Bioassay I. River Water Extracts. Water samples were collected from four study locations during the summer of 2003. Sampling sites included two Newberg locations (NP: N 45°15.567′, W 122°59.142′ and AI N 45°16.145′, W 122°59.142′), Wheatland Ferry (WF: N 45°05.447′, W 123°02.655′), and Corvallis (CV: N 44°32.887′, W 123°15.432′). On each sampling day, samples were collected from CV and one of the other three sampling locations. At each site, three 20-L grab samples were collected in stainless steel containers. Samples were typically collected at a depth of ~1 m, and containers were opened and sealed (all air removed) underwater. In all cases, collections were made at least 30 cm below the surface and at least 30 cm above the sediment. Sample extraction was completed within 96 h of sample collection.

The 60 L of water collected at each site (triplicate 20 L samples) was divided into five 12 L subsamples for extraction. Each subsample was filtered under vacuum through a 50-mm DVB-phobic followed by a DVB-phillic solid-phase extraction disk (Bakerbond Speedisk 8072-06, 8068-06, J. T. Baker, Phillipsburg, NJ). Flow rates were 15−30 mL/min. Following extraction, the disks were dried under vacuum and stored in airtight containers at −20 °C overnight. To prepare bioassay concentrates, each disk was eluted three times with 5 mL of methanol. Methanol eluents were dried by passing through a column of Na_2SO_4. For each site, dried methanol eluents were pooled and evaporated to 3 mL under a steady stream of N_2 gas using a Zymark Turbovap II. The pooled concentrates were transferred to amber glass vials and stored at −80 °C until used for bioassay.

Although not as exhaustive as multimethod procedures designed for the extraction and analysis of a wide range of organic contaminants in surface water (*45*), the extraction procedure described above was designed to capture a significant cross section of dissolved organic contaminants (log K_{ow}'s in the range of 1−7), with a resulting methanol concentrate suitable for use in a fathead minnow bioassay. Using the extraction procedure described above with ethyl acetate as the eluent, dissolved residues of over 100 current-use pesticides and POPs have been recovered and analyzed by capillary GC/MS (Usenko and Simonich, personal communication). To assess extraction efficiency, river water collected at the CV site was fortified at 0.0075 μg/L with chlorpyrifos (log K_{ow} = 4.7), a well-known Willamette River contaminant. Ethyl acetate extracts were analyzed by GC/MS using the method of Usenko and Simonich. Average recovery ± standard deviation was 91 ± 5 (n = 9).

Fathead minnows (*P. promelas*) less than 24 h post-hatch were obtained from Chesapeake Cultures (Hayes, VA). Larval fathead minnows (FHM; 24−48 h post-hatch) were randomly assigned to 400-mL beakers containing 100 mL of dechlorinated tap water (dtw). Each beaker was stocked with n = 30 larval FHM. Beakers were then randomly assigned to one of eight treatment groups. Treatment groups for the study were: control (CON; 200 mL of dtw); solvent control (SC; 0.05%

MeOH in dtw); 8X-, 4X-, and 1X-Corvallis; 8X-, 4X-, and 1X-NP, AI, or WF. 8X, 4X, and 1X represent the volume of the appropriate extract dissolved in 200 mL dtw to provide a concentration equivalent to 800, 400, and 100%, respectively, of river water concentration of the extract's constituents, assuming 100% recoveries. Methanol was added to each of the 4X and 1X treatments such that the total MeOH concentration was equivalent to that of the 8X treatments and SC (0.05%). Fifty percent of the test solution was renewed daily by drawing the solution down to 100 mL and adding 100 mL of fresh test solution containing nominal concentrations of extract, solvent, or both. The location of each beaker on the exposure bench was assigned randomly, and all beakers were aerated throughout the exposure duration.

After 5 days of exposure, surviving fish were counted and transferred to 1-L plastic containers for grow-out to ~d hb 25−30 post-hatch. During grow-out, fish were maintained in dtw supplied from a flow-through system. Throughout both exposure and grow-out, water temperatures were maintained at 24−26 °C, photoperiod was 16 h light, 8 h dark, and FHM were fed *Spiralina* (Algae Feast, Earthrise, Petaluma, CA) twice daily and brine shrimp nauplii (GSL Brine Shrimp, Ogden, UT) once daily. Dissolved oxygen, pH, ammonia, and nitrite were monitored daily.

At the end of the grow-out period, fish from each container were transferred to a 5-cm-diameter plastic tube with fine mesh at one end (PVC insert). The entire batch of live fish was immersed in a 0.2% calcein (Sigma C-0875; St. Louis, MO) solution (pH 7.0), stained for 10 min, transferred to clean water for 10 min to destain, and then euthanized by immersion in a 200 mg/L solution of MS-222 (Finquel, Argent, Redmond, WA). Euthanized specimens were immediately examined by fluorescence microscopy using a Leica MZFL111 dissecting microscope (Bartles and Stout, Bellevue, WA) equipped with a mercury lamp and fluorescein/green fluorescence protein filter. Calcein staining allows for direct visualization of calcified skeletal structures (*46*). Each specimen was examined for skeletal deformities, including scoliosis, lordosis, fused vertebrae, compressed centra, extra or missing spines, etc. Screening of several hundred fish as part of assay development confirmed that all these types of deformities were detectable by this method. Vertebral development was also scored on a scale of 1−5 using a criteria defined for this study. Digital images of each fish and close-ups of deformities, if detected, were captured and archived using ImagePro Plus 4.5.1 (Media Cybernetics, Silver Springs, MD). In some cases, examinations were spread over 2−3 d. Replicates examined each day were selected randomly.

Survival to the end of exposure (6 d post-hatch), survival to examination (28−30 d post-hatch), and percent of surviving fish with a skeletal deformity were determined for each replicate. Developmental score distributions were determined for each treatment. One-way analysis of variance was used to test for differences in survival or incidence of deformities among treatments. A nonparametric Kruskal−Wallis test on ranks was used to test treatment-related differences in developmental score distributions.

Skeletal Deformities Bioassay II. Exposure to *Apophallus donicus* Cercariae. Characterization of parasite association with vertebral deformities in Willamette River fishes was based on examination of histological sections of formalin-preserved fish as well as whole mounts of trypsin-cleared, alcian blue and alizarin red S-stained fish (*47*). The methods and statistical analysis used for the parasite characterization were reported elsewhere (*47*).

For laboratory transmission studies, laboratory-reared fathead minnows were obtained from Cheasapeake Cultures, Hayes, VA. Fish were held in dechlorinated tap water (23−26 °C) to ensure unexposed fish did not become infected. Fish were maintained in static water aquaria with biological filters. Fish were delivered at 3−7 day old and were initially fed paramecium cultures until about 10−14 days old, then were switched to a mixture of brine shrimp naupallii (GSL Brine Shrimp) and freeze-dried Spirulina algae (Algae Feast). After about 3−4 weeks, fish were then fed TetraMin flake food (Tetra Sales, Blacksburg, VA).

Fluminicola virens snails were collected from the Newberg area (Figure 1) from June to August 2003. Cercariae consistent with those described by Niemi and Macy (*48*), (Figure 2a) were harvested from individual snails by holding snails in isolation in 24-well tissue culture plates in 2 mL of water. For transmission studies, larval fathead minnows of varying age (Table 4) were exposed to known concentrations of cercariae or control water. Initial trials with very young fish resulted in high mortality in exposed fish (Table 4).

Incidence of infection, vertebral deformities, and association of worms with deformities was determined by examination of whole, preserved fish that were cleared with trypsin and stained with with alcian blue and alizarin red S (*49*−*50*). Fish were collected at either 55 or 70 days postexposure. Cleared fish were placed in a Petri dish, covered with glycerin, and examined at 25 or 50×. Fish were also evaluated by radiography, as described by Markle et al. (*14*).

Results and Discussion

Deformity Loads in Willamette River Fish. One difficulty associated with the use of fish skeletal deformities as a biomonitoring tool is the lack of information on normal background deformity rates. One study in salmonids suggested that 2−5% may be a normal background rate in wild populations (*51*); however, it is unclear whether deformity rates differ among species or among populations within species. Because background deformity rates are usually unknown, biomonitoring approaches based on skeletal deformities rely on temporal or spatial comparisons, such as year to year changes, or comparisons between locations with similar habitat, climate, etc. In Willamette River fish, the marked geographic disparity in frequency of skeletal deformities suggested localized problems at Newberg and Wheatland Ferry.

Our 2002−2003 results were consistent with previous studies that reported a greater incidence of skeletal deformities in Willamette River fish from Newberg and Wheatland Ferry relative to Corvallis (*12*, *14*). Among the five species most commonly sampled, percent frequency of deformities was generally 2−3 times greater at Newberg and Wheatland Ferry than at Corvallis (Table 1). The only exception was the large-scale sucker (*Catostomus macrocheilus*), which was the only catostomid of the five most commonly collected fishes. Among the cyprinids, mean deformity loads were usually significantly lower for Corvallis fish than for fish from Wheatland Ferry or Newberg (Table 1). There were no obvious geographic or habitat differences that explained the differences observed (*40*). Overall, biomonitoring of skeletal deformities in fish collected at different locations along the Willamette River in 2002−2003 supported the conclusion that fish populations near Newberg and Wheatland Ferry were more likely to have skeletal deformities than fish from Corvallis. Spot historical samples from museum collections confirm high precaudal deformity loads (0.33−1.54 per fish) in 1983 in Newberg and Wheatland Ferry, a lower load in 1952 (0.12) at Wheatland Ferry, and a lower load upstream of Corvallis in 1967 (0.12) (*14*). These historical data do not help us distinguish among three possibilities: (1) site variation, and the range of variation we detect is normal; (2) rates at Corvallis represent the background, and Newberg rates are elevated; and (3) rates at Newberg represent the background, and Corvallis rates are depressed.

Water Quality Characterization. Ammonia, nitrate, pH, temperature, dissolved oxygen, oxidative reduction potential,

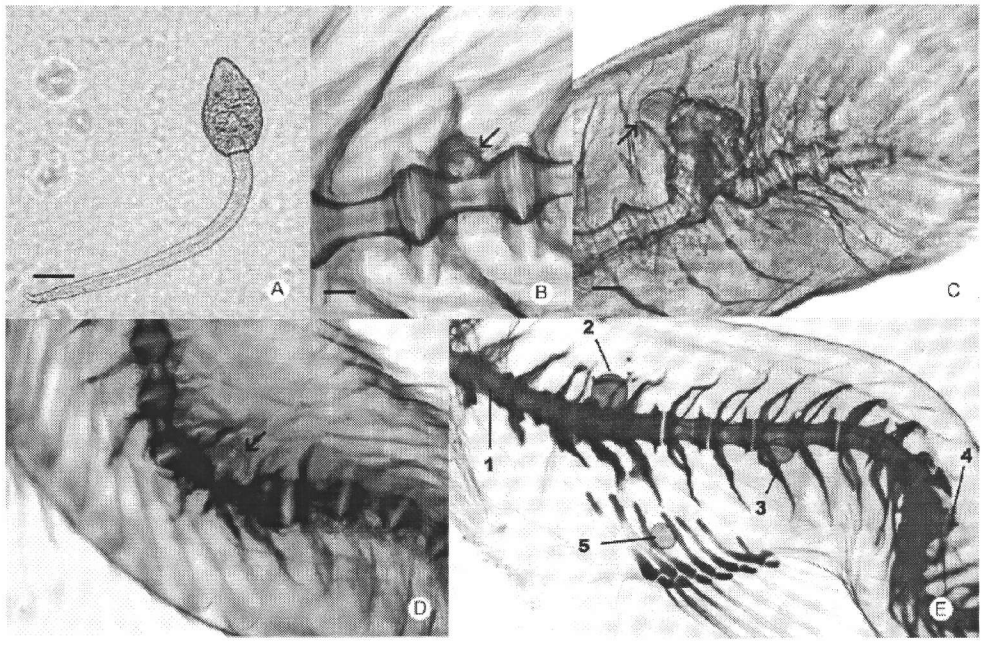

FIGURE 2. ***A. donicus*** **infections in laboratory-reared fathead minnows. A.** ***Paraplophocercous*** **cercaria from** ***F. virens*** **used in exposure trials. Bar = 50 μm. B–E. Metacercariae (arrows) associated with skeletal deformities in cleared fish. Bar = 200 μm. B. Metacercariae in surrounded by bony proliferation at base of a vertebra. C. Metacercaria in region of severe lordosis. D. Dysplastic vertebrae and lordosis at site of infection. E. Metacercariae associated with lesions: 1 and 3, no significant changes; 2, dysplastic and broken spines; 4, severe lordosis; 5, metacercariae in base of anal fin with no changes.**

TABLE 1. Frequency of Occurrence of Precaudal Deformities and Average Deformity Loads for the Five Species Most Commonly Collected from Three Willamette River Study Areas in 2002–2003

	Newberg pool			Wheatland Ferry			Corvallis		
species	%[a]	DL[b,c]	N[d]	%[a]	DL[b,c]	N[d]	%[a]	DL[b,c]	N[d]
Ptychocheilus oregonensis, northern pikeminnow	23.6	0.37 ± 0.016, A	2314	22.8	0.35 ± 0.021, A	1205	6.9	0.08 ± 0.01, B	928
Richardsonius baiteatus, redside shiner	14.2	0.24 ± 0.029, A	515	13.0	0.20 ± 0.018, A	1091	6.3	0.09 ± 0.020, B	349
Catostomous macrocheilus, largescale sucker	14.8	0.21 ± 0.015, A	1394	21.3	0.34 ± 0.111, A	47	17.3	0.22 ± 0.050, A	127
Mylocheilus caurinus, peamouth	14.1	0.20 ± 0.032, AB	305	24.2	0.34 ± 0.033, B	442	8.4	0.10 ± 0.038, A	96
Acrocheilus alutaceus, chiselmouth	39.6	0.70 ± 0.061, A	268	55.1	0.97 ± 0.103, B	109	12.8	0.17 ± 0.030, C	251

[a] Frequency of occurrence of precaudal deformities. [b] Deformity load: number of deformity categories present per individual; mean ± SE (SE is individual rather than pooled). [c] A,B,C indicate significant difference between sites ($p \leq 0.05$) based on Bonferroni multiple range test. [d] Sample size.

and specific conductance data were collected hourly during all 21-d sampling events. At each station, some parameters showed strong temporal and spatial variation, while others did not. Diurnal temperature variation was greater at the Corvallis sites (1–2 °C), than at Newberg (1 °C or less). Temperature patterns were the same for both years and were generally 12 ± 1 °C in May and rose to ~22C ± 2 °C by the end of the sampling period. Maximum pH range during 24 h, usually in late May to early June was 7.2 to 7.8 at the Newberg sites and 7.2 to 8.8 at the Corvallis sites. These large diurnal pH changes were seen in both years. At all sites, there were small decreases in pH from 2002 to 2003. Nighttime pHs were similar, but the daytime pH showed a strong geographic difference, with the Corvallis sites often 1+ pH units higher than the Newberg sites. Low pH conditions (<5.5) have been linked to skeletal deformities in suckers (*25*–*26*), but we are not aware of any reports that link pH 7.2–8.8 conditions to skeletal deformities in fish.

The oxidation reduction potential (ORP) probe tended to drift after about 7–10 days of deployment. Since all deployments were 21 days, data after 7 days were excluded from the analysis. ORP was consistently lower at the Newberg sites, as compared to the Corvallis sites. The difference ranged from <10% to a factor of 2. There was no apparent seasonality at the Corvallis sites and little difference between 2002 and 2003. There was some evidence that the ORP increased during the season and differed among years at the Newberg sites. The ORP value is a direct reading of the activity of oxidizing and reducing agents in the water, as they correspond to oxidation–reduction reactions (*52*). In general, the Corvallis

TABLE 2. Mean Concentrations (ng/L) of Bioavailable Organics Estimated from Concentrations Accumulated in Passive Sampling Devices Exposed for 21 d at Two Sites (2 locations per site) along the Willamette River[a]

	Corvallis		Newberg pool	
bioavailable	2002	2003	2002	2003
Σ PAH[c]	3.17 ± 1.47	3.22 ± 1.22	2.01 ± 0.34	1.72 ± 0.54
phenanthrene	1.15 ± 0.60	0.58 ± 0.22	0.93 ± 0.27	0.375 ± 0.107
anthracene[b]	1.32 ± 0.52	1.18 ± 0.90	0.43 ± 0.12	0.04 ± 0.02
fluoranthene[d]	0.70 ± .40	0.43 ± 0.13	0.66 ± 0.07	0.29 ± 0.07
Σ PCB[d]	0.007 ± 0.011	0.009 ± 0.004	0.013 ± 0.015	0.015. ± 0.005
Σ DDT	0.022 ± 0.028	0.036 ± 0.004	0.049 ± 0.018	0.048 ± 0.011
p,p'-DDT[c]	0.003 ± 0.005	0.011 ± 0.001	0.010 ± 0.005	0.011 ± 0.002
p,p'-DDE[c]	0.006 ± 0.008	0.016 ± 0.003	0.016 ± 0.005	0.021 ± 0.006
p,p'-DDD[d]	0.013 ± 0.019	0.010 ± 0.002	0.023 ± 0.005	0.015 ± 0.004
dieldrin[b]	0.008 ± 0.009	0.014 ± 0.002	0.023 ± 0.007	0.025 ± 0.006
chlorpyrifos	NA	0.74 ± 0.51	NA	1.38 ± 0.33

[a] Target analytes with concentrations < detection limit not shown. See Supporting Information for complete list of analytes. [b] Significant difference between sites, both years. [c] Significant difference between sites, 2002 only. [d] Significant difference between sites, 2003 only.

TABLE 3. Concentrations of Chlorinated Pesticides, Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-dioxins, and Polychlorinated Dibenzofurans Detected in Oocyte/Ovary Tissue from Northern Pikeminnow (*Ptychocheilus orgeonensis*) Collected from Newberg Pool (NP) and Corvallis (CV) Study Sites

	mean ± SE (ng/g)[a,b]		median (ng/g)[a]		
compound	NP	CV	NP	CV	P
endrin	3.94 ± 1.62	*1.62* ± 0.01	1.65	1.62	0.191
4,4'-DDD	3.63 ± 1.34	*1.62* ± 0.01	1.65	1.62	0.171
4,4'-DDE	46.9 ± 13.4	48.0 ± 15.9	36.0	31.0	0.942
PCB-8 [2,4']	3.26 ± 1.74	10.8 ± 4.68	1.65	7.70	0.144
PCB-18 [2,2',5]	*1.55* ± 0.07	16.7 ± 15.1	1.65	1.65	0.999
PCB-101 [2,2',4,5,5']	3.52 ± 0.90	*2.21* ± 0.06	4.16	1.65	0.402
PCB-110 [2,3,3',4',6]	*1.98* ± 0.46	*1.62* ± 0.01	1.65	1.62	0.674
PCB-118 [2,3',4,4',5]	*2.48* ± 0.96	*1.62* ± 0.01	1.65	1.62	0.674
PCB-138 [2,2',3,4,4',5']	*2.80* ± 1.27	*1.62* ± 0.01	1.65	1.62	0.674
PCB-153 [2,2',4,4',5,5']	4.20 ± 0.92	2.65 ± 0.65	4.20	1.65	0.163
$TEQ_{(PCDD/DFs)}$ (pg/g wet wt)	0.84 ± 0.20	0.85 ± 0.31	0.99	0.67	0.969

[a] For the purposes of calculating means, medians, and statistics, nondetects were assumed to be equal to 1/2 the method detection limit. All concentrations reported in ng/g wet wt, except TEQ, which are reported as pg/g wet wt. [b] *Italics* indicates that mean or median estimate was less than the MDL.

TABLE 4. Incidence of Vertebral Deformities and Metacercariae in Fathead Minnows (Pimephales promelas) Exposed to Cercariae of *Apophallus donicus*

trial no.	concn of exposure cercariae/fish	age of exposure (days)	days postexposure when examined	number examined	deformed %	infected %	abundance	deformities associated with parasites, %	worms associated with deformities, %
1	30	8	55	11	8/11 (73)	9/11 (82)	1.0	9/10 (90)	9/11 (82)
1C	0	8	55	7	0	0	0	NA	NA
2	10	8	70	14	12/14 (86)	13/14 (93)	1.9	17/17 (100)	20/27 (74)
2C	0	8	70	18	1/18 (6)	0	0	0	NA
3	30	5	70	10	7/10 (70)	8/10 (80)	1.2	8/9 (88)	8/12 (67)
3C	0	5	70	12	0	0	0	NA	NA
4	30	17	70	14	13/14 (93)	14/14 (100)	4.5	31/33 (94)	38/64 (59)
5	30	24	70	21	19/21 (91)	20/21 (95)	4.0	37/42 (88)	58/88 (66)
4/5C	0	17/24	70	18	1/18 (6)	0	0	0	NA
	total exposed			70	84	91	2.5	93	66
	total controls			56	4	0	0	0	0

waters were more oxidizing, or less reducing, than Newberg waters. ORP is known to influence microbial growth, but it is not clear whether the difference in ORP observed would make Newberg fish more vulnerable to infection by microbial agents or more susceptible to deformities.

Specific conductivity (SC) was very similar at the Corvallis and Newberg sites, with both showing a slight increase from May to July. The SC pattern was similar for both years. The dissolved oxygen (DO) pattern was similar at both sites. A diurnal pattern was apparent, and a slight decrease in DO was seen from May to July in both 2002 and 2003. A seasonal increase in ammonia from 0.05 to 0.25 mg/L was measured at both sites, with Corvallis generally having higher ammonia than Newberg. The nitrate probe was not robust. In 2002, drift generally occurred within 24 h of deployment. In 2003, the probe failed within a few hours of deployment. The limited data indicated that nitrate increased from May to July, and concentrations were higher at the Newberg sites, as compared to the Corvallis sites. As a whole, water quality monitoring provided no compelling evidence to suggest that differences in nutrient concentrations, pH, temperature, DO, or specific conductivity were likely causes of the different deformity

loads observed at Newberg versus Corvallis, but further investigation of a possible link between ORP differences and susceptibility to infection may be warranted.

Bioavailable Contaminants. *Polycyclic Aromatic Hydrocarbons (PAHs).* Total bioavailable PAHs were low at all locations and for all sampling events (generally < 4 ng/L). Of the 16 PAHs measured, 13 PAHs were below detection limits (< 0.1 ng/L) in 2002, and 10 PAHs were below in 2003. Three PAHs—phenanthrene, fluoranthene, and anthracene—were detected at sites in 2002 and 2003. At the Corvallis sites, phenanthrene and anthracene were equally abundant in 2002, whereas fluoranthene was somewhat less abundant (Table 2). Of the three PAHs detected at the Newberg sites in 2002, phenanthrene was the most abundant (Table 2). In 2003, anthracene was most abundant at Corvallis, and phenanthrene was most abundant at Newberg of the 16 individual bioavailable PAHs. In 2002, the Corvallis sites consistently had higher total PAH (ΣPAH) concentrations, as compared to the Newberg sites (Table 2). The average ΣPAH in 2002 was ~3.2 ng/L at Corvallis, whereas the Newberg sites had ΣPAH concentrations in 2002 of about ≤ 2 ng/L. In 2003, this pattern was again upheld (Table 3). The Corvallis stations (1 and 2) had ΣPAHs of 3.9 and 2.7 ng/L respectively; the Newberg stations (1 and 2) had ΣPAHs of ≤2 ng/L. Phenanthrene produces spinal curvature in zebrafish embryos at concentrations near water saturation (1.25 mg/L) (*53*). With low concentrations detected at both sites, PAHs were not considered to be likely contributors to the difference in deformity loads associated with the two areas.

Polychlorinated Biphenyls (PCBs). PCB analysis for this study was based on a congener-specific approach; however, concentrations of all congeners were quite low, so interpretation of results focused on total PCB concentrations. The total bioavailable PCB concentrations were generally very low, < 0.03 ng/L, at all sites, and many sites were below detection limits (0.001 ng/L) (Table 2). During 2002 and 2003, total bioavailable PCBs were generally greater at the Newberg sites (Table 2). However, since most of the data were below or near detection limits, it was difficult to draw any firm conclusions. PCB concentrations were approximately 500-fold lower than the chronic ambient water quality criteria, (CCC) of 0.014 μg/L (*54*) and they did not readily explain the difference in deformity loads among sites.

Pesticides. The bioavailable ΣDDT (sum of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE) concentrations were low (generally <0.07 ng/L) at all sites in 2002 and 2003 (Table 2). ΣDDT concentrations were ~10-fold below the freshwater CCC, 0.001 μg/L (*54*). The differences in ΣDDT between Newberg and Corvallis sites in 2002 ($p = 0.18$) and in 2003 ($p = 0.065$) were not significant (Table 2). Interannual differences in ΣDDT among stations were not significant ($p = 0.49$ Newberg; $p = 0.24$ Corvallis). In 2002, the DDT profile was dominated by *p,p*-DDD, followed by DDE and DDT (Table 2), as would be expected from older deposits. However, in 2003, DDE dominated, followed by DDD and DDT (Table 2). DDD and DDE concentrations detected at Newberg sites in 2002 and 2003 were not significantly different ($p = 0.18$). DDE concentrations but not DDD concentrations at Corvallis sites increased significantly between 2002 and 2003 ($p = 0.041$ DDE; $p = 1$ DDD). Mean DDT concentrations at Newberg did not increase significantly between 2002 and 2003 ($p = 1$; Table 2), but mean bioavailable DDT detected at Corvallis increased 4-fold ($p = 0.004$; Table 2). The cause of the increased *p,p'*-DDT concentration was not clear, but the results suggest possible new inputs or sediment disruption remobilizing DDT. Overall, the concentration differences observed were very small (≈0.008 ng/L), so conclusions should be drawn prudently.

Dieldrin concentrations were slightly higher at the Newberg sites, as compared to the Corvallis sites (Table 2; 2002 $p = 0.015$; 2003 $p = 0.004$). However, the bioavailable concentrations of dieldrin were very low, < 0.03 ng/L, and there was no significant difference between years (Newberg $p = 0.394$; Corvallis $p = 0.818$; Table 2). The dieldrin concentrations detected were around 2000-fold below the freshwater CCC of 0.056 μg/L (*54*) and are well below those generally thought to be toxic to fish (*55*−*56*). We are not aware of any reports linking below nanogram-per-liter concentrations of dieldrin to skeletal deformities.

Chlorpyrifos was the only organophosphate pesticide detected. Dimethoate, diazinon, and azinphos-methyl were below detection limits at all sites for all sampling events (detection limits were estimated at 2, 3, and 2 ng/L, respectively). In 2003, the estimated, bioavailable, water concentration for chlorpyrifos averaged 0.74 ± 0.51 at Corvallis and 1.38 ± 0.33 ng/L at Newberg (Table 2). There was little difference between the sites in June and July, but there was some difference between the sites in May, with chlorpyrifos concentrations at the Newberg sites higher than at the Corvallis sites. However, the difference was not significant ($p = 0.33$). The bioavailable chlorpyrifos concentrations observed in this study were below the chronic aquatic life criteria of 0.04 μg/L and well below concentrations reported to be toxic to fish (*57*). Observed chlorpyrifos concentrations are also well below the 95% confidence lower limit of the benchmark concentration estimate of 0.015 μg/L resulting in 2.5% brain acetylcholinesterase (AChE) inhibition in steelhead trout (*58*). Brain AChE is considered as a sensitive indicator of sublethal effects. Additionally, we are unaware of any peer-reviewed reports linking lesser concentrations of chlorpyrifos to skeletal deformities in fish.

Metals. Bioavailable heavy metal samples were collected using diffusive gel thin films (DGT), and samples were deployed in the same manner as the other PSDs. Bioavailable zinc, cadmium, lead, and copper were determined for all six sampling events. Zinc, cadmium, lead, copper, and arsenic (III) were determined in filtered grab water samples pulled during the 2003 sampling deployments. The bioavailable metal concentrations were, for the most part, below detection limits. The only exceptions were detection of ~100 μg/L of Zn in a single sample from Newberg and 7 μg/L of Pb in a single sample from Corvallis. Neither of these detections were replicated, and all other samples from both sites were below detection limits, so both detections were considered artifacts. The results provided no evidence that bioavailable heavy metals were a likely cause for the difference in deformity loads at the two sites.

Maternal Transfer of POPs. Concentrations of POPs detected in Willamette River northern pikeminnow ovary/oocyte tissue were relatively low. Chlorinated pesticide concentrations were generally < 3.3 ng/g wet wt, and only 3 of the 21 different chlorinated pesticide residues analyzed were detected (Table 3). Of the three, 4,4'-DDE was detected with the greatest frequency and at the greatest concentrations (Table 3). Endrin and 4,4'-DDD were detected in 2 of 10 samples analyzed, both from Newberg fish. Exposure to parts-per-billion concentrations of toxic chlorinated pesticides similar to those detected in the ovary/oocyte tissue from some of the fish analyzed have been shown to cause adverse effects in early life stage fish (*59*−*61*). Thus, some potential for toxic effects of maternally transferred chlorinated pesticides was possible, although more extensive study would be needed to determine how probable such effects are.

Concentrations of maternally transferred PCBs detected in northern pikeminnow ovary/oocyte samples were not alarming. A total of seven different PCB congeners were detected in one or more of the samples (Table 3). PCB 153 was detected the most frequently (6/10 samples) and at the greatest concentration, (up to 11.2 ng/g; Table 3). PCBs 110, 118, and 138 were each detected in a single Newberg fish.

All PCB congeners detected were mono- or diortho-substituted. These congeners tend to be much less toxic than nonortho planar PCBs (*59*, *62*, *63*). Fish, in particular, have been shown to be less sensitive to the mono-ortho PCBs than mammals or birds (*63*−*64*). It has even been suggested that coexposure to the relatively nontoxic mono- and diortho PCB congeners may reduce the overall uptake of the more toxic nonortho planar PCBs (*59*). Mean concentrations of PCBs 8 and 18 were greater in fish collected from Corvallis than from Newberg (Table 3). Conversely, the mean concentration of PCB 153 in ovary/oocyte tissue was greater in Newberg fish (Table 3). However, neither of these differences was significant. On the basis of the samples analyzed, maternal transfer of PCBs does not appear to pose a high risk of overt early life stage toxicity to Willamette River northern pikeminnow.

PCDDs and PCDFs were detectable in all ovary/oocyte samples. Specific congeners varied considerably among samples; therefore, a toxic equivalents approach (*64*) was used to facilitate analysis of the results and comparison among sites. Total 2,3,7,8-TCDD equivalents in oocyte/ovary tissue of Willamette River northern pikeminnow ranged from 0.18 to 2.06 pg/g wet wt. The greatest TEQ concentration (2.06 pg/g wet wt) was detected in a fish from Corvallis; however, the mean TEQ concentrations were nearly identical for fish collected from the two study sites (0.84 ± 0.20 versus 0.85±0.31 pg/g wet wt for Newberg and Corvallis, respectively; Table 3). Total concentrations of TEQs were less than those expected to cause toxicity during early life stage development. On the basis of measured TCDD concentrations in fish eggs, the lowest observed effect concentration for seven different fish species ranged from 270 to 2000 pg/g wet wt (*65*). NOECs were > 175 pg/g wet wt (*65*). The TEQ concentrations detected in this study were at least 135 times lower than the LOEC for the most sensitive of the seven species tested (*65*). Furthermore, the TEQ concentrations detected were at least 2.5 times less than the probable no-observable-adverse-effect level (NOAEL) of TEQs for lake trout, which is widely regarded as the fish species most sensitive to dioxin and dioxin-like toxicity (*65*, *66*). Thus, concentrations of maternally transferred PCDDs and PCDFs did not appear likely to cause early life stage mortality.

On the basis of the literature, it was unclear whether any of the concentrations of POPs detected in Willamette River northern pikeminnow ovary/oocyte tissue would be likely to cause skeletal deformities. However, no significant differences in maternally transferred POP concentrations were observed for fish from the Newberg versus Corvallis sites (Table 3). Even if early life stage exposure to POPs was causing some disruption of early development, leading to skeletal deformities, it was unlikely to account for 2−3-fold greater rates of skeletal deformities. As a whole, these results provided no compelling evidence to support the hypothesis that greater maternal transfer of POPs was a likely cause for the greater incidence of skeletal deformities in fish from the Newberg region of the Willamette River.

Sediment POPs. A small number ($n = 3$ per site, per year) of surficial sediment samples collected from Newberg and Corvallis sites were analyzed for persistent chlorinated pesticide residues and PCBs to determine whether trophic transfer of these compounds from sediment, or direct exposure of embryo-larval fish (particularly for broadcast spawners; (*40*)) could account for differences in deformities at the two sites. Chlorinated pesticides were not detected in samples collected from Corvallis or Newberg in either 2002 or 2003. In 2002, PCB 8 was detected in 2/3 Corvallis samples and 1/3 Newberg samples. Concentrations of PCB 8 ranged from 1.3 to 6.6 ng/g. Additionally, PCB 128 was detected in a single Corvallis sample and PCBs 18, 101, and 153 were detected in a single Newberg sample. Concentrations of these congeners ranged from 0.5 (PCB 101) to 3.8 (PCB 18). In 2003, only 2 congeners, PCB 101 and PCB 110, were detected. PCB 101 was found in one Corvallis sample and two Newberg samples at concentrations ranging from 0.37 to 1.2 ng/g. PCB 110 (1.1 ng/g) was detected in a single Corvallis sample. Samples were not collected at identical locations each year, so it was not possible to determine whether differences in the congeners detected were the result of spatial or temporal differences. Overall, the results did not provide compelling support for the hypothesis that chlorinated pesticide residues or PCBs present in surficial sediments were a likely cause for the greater skeletal deformity load in Newberg fish.

Skeletal Deformities Bioassay I: River Water Extracts. Results of laboratory exposure of fathead minnows to Willamette River water extracts from d 2 to d 6 post-hatch with subsequent grow-out to d 28−30 post-hatch did not provide evidence that unknown compounds or interactions between chemicals present in the prepared extracts were likely causes of greater deformity loads observed in fish from certain regions of the Willamette River. Survival to d 6 post-hatch ranged from 83 to 100% in all trials, and there were no significant differences among treatments ($p = 0.202$−0.754), indicating that extracts were not acutely toxic to larval fathead minnows. Survival during grow-out was variable among replicates and among trials, ranging from 5 to 19 fish per replicate (17−63%). In all cases, a minimum of 20 fish per treatment group were examined for deformities. It was not possible to determine whether fish that died during grow-out were deformed. Nonetheless, the lack of a significant treatment-related effect on survival to the examination day ($p = 0.425$−0.930) suggests that the mortality during grow-out was randomly distributed among replicates and did not obscure a treatment effect.

When simple dorsal−ventral curvature was included as a deformity, 5−25% of the fish examined were classified as deformed, although no treatment-dependent effect was observed ($p = 0.834$−0.929). When the analysis was restricted to only those deformities characterized as "qualitatively similar to those observed in Willamette River fish" (as per categories defined by Cunningham et al. 2004 (*40*)), the incidence of deformities ranged from 0.8%-2% for the entire population surveyed in each trial. Given total sample sizes of 210−397 fish per trial, this represented 2−8 fish. In all cases, deformities were spread across treatments, such that no association with any particular treatment was evident.

The distribution of developmental scores was unaffected by treatment in the NP/CV and AI/CV trials ($p = 0.255, 0.470$), with most fish having developmental scores greater than 3. In the WF/CV trial, fish from the 4XC group were significantly more developed than those from all other treatment groups ($p = 0.024$). However, no concentration-dependence was evident. As a whole, there was no evidence that Willamette River water extracts induced skeletal deformities or otherwise adversely affected larval fathead minnows exposed for 96 h from d 2 to d 6 post-hatch.

It must be noted, however, that a negative response in the skeletal deformities bioassay did not rule out the possibility that chemicals contained in the extracts had the potential to induce deformities in cyprinid fish. As designed, the assay was able to provide a reasonable screen for the potential of the river water extract to disrupt some early life-stage developmental processes important for formation of the ossified vertebral column. The assay was not expected to be an effective screen for chemicals able to cause skeletal deformities through acute neuromuscular damage. A time-series for skeletal development in FHM held under assay conditions showed that as early as d 5 post-hatch, nearly all fish had ossified skulls and partial vertebral formation, as indicated by ossification of the anterior-most centra (unpublished results). Attempts to validate the assay using Cd,

Se, and chlorpyrifos as positive controls were unsuccessful, as deformities were not induced at nontoxic concentrations (unpublished results). Robust application of the method will require additional characterization of the detectable mechanisms of action, and further optimization to reduce mortality-related variability during grow-out.

Association of Parasites with Deformities in Field Collected Fish. The occurrence of skeletal deformities in Willamette River fish was strongly linked with metacercariae of a digenean trematode, likely *Apophallus donicus* (*40, 47, 48*). An analysis of cleared and stained specimens of northern pikeminnow and chiselmouth collected from four Willamette River locations, including Newberg, Wheatland Ferry, and Corvallis (Figure 1), concluded that the probability of having a precaudal skeletal deformity was strongly dependent on the number of trematode cysts in the body ($p < 0.0001$) and the location in the river ($p = 0.006$) (*40*). Species and fish size were not significant predictors (*40*). Trematodes were directly associated with 86.5% of 592 primary precaudal deformities detected in chiselmouth and 46.3% in northern pikeminnow (*40, 47*). Additionally, a *Myxobolus* sp., likely *Myxobolus cyprini*, was associated with a significant percent (36%) of northern pikeminnow with histologically verifiable skeletal deformities (*47*). These results suggested that parasites were a likely cause for the skeletal deformities observed in Willamette River fish. However, solely on the basis of examination of field collected specimens, it was not possible to determine whether parasites were actually causing deformities or whether deformed fish were simply more vulnerable to infection.

Skeletal Deformities Bioassay II. Exposure to *Apophallus donicus* Cercariae. Results of the laboratory infection studies convincingly demonstrated that vertebral deformities consistent with those observed in Willamette River fish could be caused by trematode cercariae identified as *Apophallus donicus* (Figure 2). Five separate exposure trials were conducted with fathead minnows (a cyprinid species) ranging from 8 to 24 days old (post-hatch). Mortality was variable and often high in both cercariae-exposed (14−71%) and control fish (5−91%). Nonetheless, conclusions could be drawn. A high incidence of infection (80−100%) was observed in cercariae-exposed fish from all trials, and infected fish exhibited a high incidence of vertebral deformities (70−93%; Table 4). Most deformities were directly associated with metacercariae (Figure 2), and nearly all trematodes were directly located along the vertebral column. The types of deformities observed were also identical to those observed in field-collected specimens (*40, 47*) including extra spines, lordosis, fused vertebrae, and increased vertebral density (Figure 2). As in field-collected specimens, metacercariae occurred directly appressed to or deep within vertebrae and were often associated with bone hypertrophy. In contrast to cercariae-exposed fish, only 4% of the control fish examined exhibited skeletal deformities (Table 4). Control deformities were characterized as curvature of the spine or fused vertebrae. The incidence of skeletal deformities in control fish was similar to background rates of skeletal deformities determined for lab-reared fathead minnows examined by fluorescence microscopy as part of exposures to river water extracts.

Controlled laboratory exposure to *A. donicus* replicated vertebral deformities observed in fish collected from the field and further demonstrated that this parasite was likely a major cause of deformities in Willamette River cyprinid fish. This heterophyid digenean trematode exhibits broad host specificity, infecting many species in the family Cyprinidae as well as fish from several other families (*48*). As observed in both our laboratory and field studies, the parasite exhibits remarkable affinity for bone (*40, 47*). Most of the metacercariae were associated directly with skeletal structures and were not found in the viscera. Similar to *Apophallus* sp. in the present study, *A. brevis* in yellow perch apparently does not infect the visceral organs (*67*). Taylor et al. (*68*) described bony ossicles in yellow perch caused by *A. brevis*. Infections by other metacercariae types have been linked to vertebral anomalies. Muscle infections by *Bucephalus polymorphus* caused vertebral deformities in cyprinid fishes (*69*), and *Riberiora sp.* was suspected to be a major cause of supernumerary limbs and other vertebral changes seen in North American frogs (*70*). Thus, both empirical evidence and literature reports support the conclusion that trematode parasites are causing skeletal deformities in Willamette River fish.

Future Investigation. Although parasitic infection is likely the primary cause of skeletal deformities in Willamette River fishes, questions remain as to whether spatial differences are due to natural or anthropogenic factors. Increased occurrence of trematode infections have been linked to anthropogenic pollution and physical alteration of aquatic habitats caused by human activities (*71*). Potential synergism between exposure to herbicides and pesticides and susceptibility of frogs to infection by metacercariae of *Ribeiroia sp.* and *Telochris sp.* have also been reported (*72*). None of the chemical contaminants detected in this study are known to cause immune suppression or increase susceptibility to infection at the concentrations observed. However, the biological assays used in this study did not test the interaction between exposure to parasites and exposure to complex mixtures of chemicals present in Willamette River water or sediment extracts. This would be a useful step toward determining whether these chemicals promote susceptibility to parasites. Alternatively, it is possible that spatial differences in deformities reflect a natural phenomenon. Given the life cycle of *Apophallus donicus* (*48*), any habitat characteristics favoring either the intermediate host (snails such as *Fluminicola virens*) or the definitive host (fish-eating birds) could result in greater *Apophallus sp.* abundance and potentially more infections. Natural factors, influencing the viability and numbers of microbial and other infectious agents, such as ORP, may also play a role. Additional study parasite ecology and potential interactions with anthropogenic influences could help determine appropriate management actions for affected regions of the Willamette River basin.

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Supporting Information Available

(1) List of all target analytes and parameters analyzed as part of in situ monitoring with a YSI 6920 Sonde probe and in situ sampling of bioavailable organic compounds and metals using PSDs and DGTs, (2) diagram of float-cable-cage-cable-anchor setup, (3) 2002 and 2003 pH and ORP trends, (4) list of target analytes analyzed in ovary/oocyte tissues and surficial sediments and the concentrations detected in each sample, (5) developmental scoring criteria used for fathead minnow skeletal deformities assay, and (6) examples of deformities observed in fathead minnow skeletal deformities assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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