Biomarkers Signal Contaminant Effects on the Organs of English Sole (*Parophrys vetulus*) from Puget Sound

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Fish living in contaminated environments accumulate toxic chemicals in their tissues. Biomarkers are needed to identify the resulting health effects, particularly focusing on early changes at a subcellular level. We used a suite of complementary biomarkers to signal contaminant-induced changes in the DNA structure and cellular physiology of the livers and gills of English sole (*Parophrys vetulus*). These sediment-dwelling fish were obtained from the industrialized lower Duwamish River (DR) in Seattle, Washington, and from Quartermaster Harbor (QMH), a relatively clean reference site in south Puget Sound. Fourier transform–infrared (FT-IR) spectroscopy, liquid chromatography/mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS) identified potentially deleterious alterations in the DNA structure of the DR fish livers and gills, compared with the QMH fish. Expression of CYP1A (a member of the cytochrome P450 multigene family of enzymes) signaled changes in the liver associated with the oxidation of organic xenobiotics, as previously found with the gill. The FT-IR models demonstrated that the liver DNA of the DR fish had a unique structure likely arising from exposure to environmental chemicals. Analysis by LC/MS and GC/MS showed higher concentrations of DNA base lesions in the liver DNA of the DR fish, suggesting that these base modifications contributed to this discrete DNA structure. A comparable analysis by LC/MS and GC/MS of base modifications provided similar results with the gill. The biomarkers described are highly promising for identifying contaminant-induced stresses in fish populations from polluted and reference sites and, in addition, for monitoring the progress of remedial actions.
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The contamination of coastal environments poses an obvious threat to the health of a broad spectrum of phylogenetically diverse organisms. Consequently, a keen interest exists in finding ways to assess the health impacts on exposed populations of aquatic species (Barron et al. 2002; Myers et al. 1991). Biologic indicators play a prominent role in the assessment of toxic chemical exposures and effects (Ostrander 2005). One goal is the development of biomarkers for determining whether xenobiotics have altered the health of aquatic species at a contaminated site and whether remedial action (e.g., removal of contaminated sediments) has proven successful in reducing observed toxic effects (Cajaraville et al. 2000).

In an early discovery, Dawe et al. (1964) found neoplasia in the livers of wild fish from polluted sites. This discovery focused considerable attention on the health status of fish living in these environments. Subsequently, environmental chemical exposures were linked to other cellular lesions in fish livers (e.g., megalocytic hepatosis and foci of cellular alteration) (Malins et al. 1987; Moore and Myers 1994; Myers et al. 1991).

Refractory chemicals [e.g., polychlorinated biphenyls (PCBs)] that accumulate in the livers of fish produce hepatocellular carcinomas in rodents (Mayes et al. 1998). Polynuclear aromatic hydrocarbons (PAHs) (e.g., benzo[*a*]pyrene), which are readily metabolized in the livers of fish to carcinogenic metabolites, have been putatively linked to the development of liver tumors (Maccubbin 1994). Moreover, a number of subcellular alterations, such as in gene expression (Peterson and Bain 2004; Roling et al. 2004), and changes in immune response (Grinwis et al. 2000; Mondon et al. 2000) result from the intake of PAHs, PCBs, and other toxic organic compounds.

Field studies on the effects of sediment contamination on bottom-dwelling English sole (*Parophrys vetulus*) at multiple Puget Sound sites (Washington State) were conducted between 1979 and 1985. These studies revealed significantly higher concentrations of toxic chemicals in Seattle's industrialized Duwamish River (DR), notably PAHs and PCBs, compared with "clean" reference sites (e.g., Case Inlet) (Malins et al. 1987). Hepatic neoplasms (~ 17% prevalence) were revealed in fish from urban (industrialized) sites, such as the DR, but not in fish from nonurban (essentially nonindustrialized) reference sites (Malins et al. 1987). Noncancerous idiopathic lesions (e.g., foci of cellular alteration) were found primarily in fish from the DR, compared with the reference sites (Malins et al. 1987). In 2001, the state of the environment in the DR caused the U.S. Environmental Protection Agency (EPA) to add the Lower Duwamish Waterway to the National Priorities List (Superfund) because of sediment contamination (U.S. EPA 2001).

Factors giving rise to differences in biologic effects between the DR and reference sites are obviously complex and multifaceted. Reactive oxygen species [ROS; e.g., the hydroxyl radical ('OH)] likely contributed to these differences by reacting with and thus damaging the DNA bases of fish exposed to toxic chemicals (Malins et al. 1996). This damage has been associated with an increased risk for tumor formation. For example, a previous comparative study of liver DNA in English sole from the DR in 1993 and 1995 and in Quartermaster Harbor (QMH), a relatively clean location in south Puget Sound, in 1995 showed higher concentrations of the mutagenic base lesions 8-hydroxyguanine (8-OH-G) and 8-hydroxyadenine (8-OH-A) in the DR fish (Malins et al. 1996).

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