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Ecological Effects of *In Situ* Sediment Contaminants

Sediment-associated contaminants and liver diseases in bottom-dwelling fish

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Abstract

High concentrations of chemicals have been found in sediments from urban areas of Puget Sound. Hundreds of organic chemicals (including certain aromatic hydrocarbons [AHs] and various chlorinated compounds) were identified. Statistical methods were used to evaluate possible relationships between the chemistry data and fish diseases. Positive correlations were found between the frequencies of liver neoplasms (e.g., hepatocellular carcinoma) and other liver lesions in English sole (*Parophrys vetulus*) and concentrations of AHs in sediment; such correlations were not found with chlorinated hydrocarbons. Strong evidence was also obtained to show that many organic chemicals in sediment are bioavailable to bottom-dwelling fish. Stomach contents (consisting mainly of benthic invertebrates) from English sole had concentrations of a number of AHs similar to those in the sediment from which the fish were taken. In these same fish, metabolites of many aromatic compounds were found in bile using a procedure combining HPLC with fluorescence detection. Further, the concentrations of certain xenobiotic metabolites in bile were correlated positively with the occurrence of liver neoplasms in English sole.

Introduction

High prevalences of neoplasms in bottom fish from polluted waters of the United States have been described in the recent literature. These conditions have been reported, for example, in freshwater fish from Torch Lake, Michigan (Black *et al.*, 1982); the Niagara River, New York (Black, 1983); rivers associated with the Great Lakes (Baumann *et al.*, 1982); and in demersal saltwater fish from urban areas of Puget Sound, Washington (McCain *et al.*, 1982; Malins *et al.*, 1982, 1984). Although these studies provide strong evidence of a connection between hepatic lesions in fish and pollution, little evidence exists about relationships between these diseases and specific chemicals or groups of chemicals. The present paper reviews findings from our laboratory on relationships between sediment chemicals and prevalences of liver neoplasms in bottom fish from Puget Sound (Fig. 1). These findings suggest that aromatic hydrocarbons (AHs) may play a role in the etiology of hepatic neoplasms in bottom fish. In addition, we will report our most recent research results which provide supportive evidence for these findings.

Sediment chemicals and diseases of bottom fish from Puget Sound

In most of our Puget Sound pollution studies (McCain *et al.*, 1982; Malins *et al.*, 1982, 1984), we focused on diseases of English sole (*Parophrys vetulus*), rock sole (*Lepidopsetta bilineata*) and Pacific staghorn sculpin (*Leptocottus armatus*). The organs of fish containing the greatest numbers of lesions of all types were the liver, kidney, and gills. Some lesions were associated with parasites or

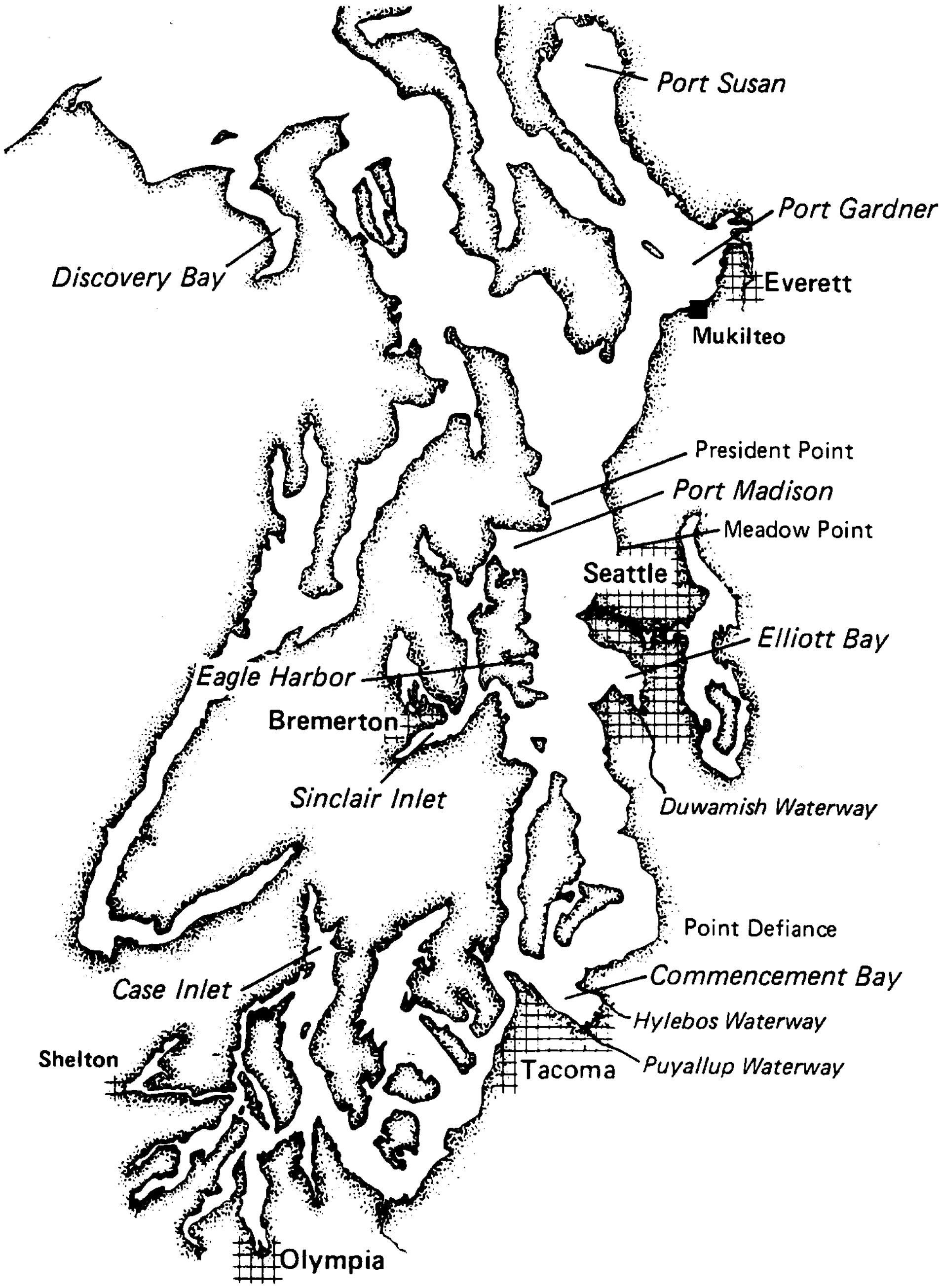

Fig. 1. Map of Puget Sound, Washington, showing locations of sites sampled.

microorganisms, but other lesions (idiopathic) had no such apparent association. Idiopathic lesions were found frequently in the liver. Neoplasms – liver cell adenoma, hepatocellular carcinoma (Fig. 2), cholangiocellular carcinoma, hemangioma and fibroma – constituted a major type of liver lesion. Other major types of idiopathic liver lesions were hyperplasia/foci of cellular alteration [which have been reported to be 'preneoplastic' in laboratory rodents (Firth & Ward, 1980)], megalocytic hepatosis, and steatosis/hemosiderosis (McCain *et al.,* 1982). Fish with hepatic neoplasms

Fig. 2. Liver with multiple neoplastic nodules (arrows) from an English sole from Puget Sound, Washington.

were primarily from urban-associated waters. No tumor-bearing fish (156 examined) were found in nonurban waters during 1979 to 1982; however, recent samplings conducted in 1983 and 1984 have found tumor-bearing English sole in Port Madison and Case Inlet. High prevalences of hepatic neoplasms were found, for example, in English sole from the Duwamish Waterway in Seattle: 8.2% (n = 537), from the Hylebos Waterway in Tacoma: 3.4% (n = 297) and from near Mukilteo: 7.5% (n = 66) (Malins *et al.,* 1984).

A great variety of chemicals were identified in Puget Sound and the highest concentrations were found in urban-associated sediments (Malins *et al.,* 1982, 1984). For example, the mean concentration of polychlorinated biphenyls (PCBs) in sediment was more than 100 times greater in Tacoma's Hylebos Waterway (Table 1) than in sediment from the

Table 1. Concentrations of selected xenobiotics in sediments from four urban and two nonurban areas in Puget Sound.

Chemical	Concentration (ppm)					
	Duwamish Waterway[1] (n = 5)	Hylebos Waterway[1] (n = 4)	Mukilteo[2] (n = 2)	Eagle Harbor (n = 3)	Port Madison[1] (n = 2)	President Point[2] (n = 1)
Polychlorinated biphenyls	0.36 ± 0.21	0.53 ± 0.47	0.11 ± 0.12	<0.01	<0.01	<0.01
Aromatic hydrocarbons	14 ± 9.4	17 ± 20	25 ± 24	142 ± 55	0.48 ± 0.24	1.1
Phenanthrene	1.2 ± 0.84	2.0 ± 3.0	2.4 ± 2.6	13 ± 9.1	0.04 ± 0.02	0.15
Anthracene	0.57 ± 0.36	0.24 ± 0.26	0.36 ± 0.22	14 ± 0.58	<0.01	0.15
Pyrene	1.7 ± 1.1	2.2 ± 2.7	2.8 ± 1.8	27 ± 19	0.07 ± 0.04	0.09
Benz[a]anthracene	1.3 ± 0.78	1.2 ± 1.3	0.83 ± 0.53	8.0 ± 6.9	0.05 ± 0.03	0.07
Benzo[a]pyrene	0.66 ± 0.50	0.57 ± 0.68	0.36 ± 0.27	3.1 ± 2.0	0.02 ± 0.01	0.04

[1] Malins *et al.*, 1982.
[2] Malins *et al.*, 1985b.

nonurban area of Port Madison. Mean concentrations of AHs were as much as 50 times greater in parts of Elliott Bay, compared to nonurban areas. High concentrations of certain polynuclear aromatic hydrocarbon carcinogens, including benz[a]anthracene (BaA) and benzo[a]pyrene (BaP), were found in sediments with the highest concentrations of AHs (Table 1). Over 900 individual organic compounds were detected in Commencement Bay (Tacoma) sediments. These included more than 500 AHs, hundreds of chlorinated hydrocarbons, and various bromine-, sulphur-, nitrogen- and oxygen-containing compounds. The numbers and identities of these compounds have not been fully determined because of the complexity of the chemical mixtures and the lack of chemical standards. Generally, mean concentrations of sediment-associated metals (e.g., lead) were higher in the urban areas (Sinclair Inlet, Commencement Bay and Elliott Bay) than in the non-urban areas. The mean concentrations of cadmium, however, were similar in urban and nonurban sediments.

We applied mathematical/statistical analyses to relate chemicals in Puget Sound sediments with prevalences of hepatic lesions in English sole. Factor analysis, a mathematical method for grouping chemicals whose concentrations in sediments correlated positively with each other, was applied to the sediment chemistry data (Malins *et al.*, 1984). Four major factors were obtained, which were dominated by AHs, metals, PCBs, and other chlorinated hydrocarbons, respectively. Using the Spearman's rank correlation coefficient procedure, the prevalences of hepatic neoplasms and various non-neoplastic liver disorders correlated positively ($p < 0.003$) with sediment concentrations of AHs (Table 2). Significant positive correlations were not obtained with the chlorinated organic compounds.

Although statistically significant correlations between AHs in sediment and fish lesions demonstrated in this study should not be considered as

Table 2. Spearman's rank correlation coefficients (r_s) and significance levels for prevalences of hepatic lesions in English sole and chemical concentrations in bottom sediment (from Malins *et al.*, 1984).

Lesion type	Chemical group[1]	r_s	Significance level
Neoplasms	AHs	0.48	0.003
Hyperplasia/FCA	AHs	0.47	0.004
Megalocytic hepatosis	AHs	0.54	0.001
Steatosis/hemosiderosis	AHs	0.49	0.002
One or more hepatic lesions	AHs	0.58	0.001
One or more hepatic lesions	Metals	0.54	0.001

[1] Chemical groups selected by factor analysis included: aromatic hydrocarbons, metals, selected metals plus PCBs, and chlorinated compounds.

direct evidence of specific cause-and-effect, they do have considerable value for identifying presumptive relationships between sediment-associated chemicals and lesions of fish in spite of the tremendous complexity of the marine environment. Due to this complexity, the results of this study had several limitations. One limitation concerned the bioavailability of sediment-associated AHs and other carcinogenic chemicals (e.g., nitrogen-containing hetrocycles).

Accordingly, our recent studies of Puget Sound have focused on both the transport of chemicals from sediments to organisms and on factors that govern the subsequent transfer of chemicals through food chains (Malins *et al.,* 1985a).

Bioavailability of sediment-associated AHs

AHs were usually present in only trace amounts in livers of English sole from polluted areas, even when the sediments contained high concentrations of AHs. However, when we examined the stomach contents (consisting mainly of Annelida) of English sole from Mukilteo we found concentrations of AHs, such as BaA and BaP (1000 and 570 ppb, respectively), similar to those in the sediment (Table 1) from which the fish were taken (Malins *et al.,* 1985b). The low accumulations of AHs in fish liver can be explained by the fact that AHs are extensively converted in this organ to metabolites (Roubal *et al.,* 1977; Varanasi & Malins, 1977; Varanasi *et al.,* 1979; Gruger *et al.,* 1981; Malins & Hodgins, 1981; Varanasi & Gmur, 1981a, b), which are not routinely detected even by the most sophisticated techniques available.

Other recent studies demonstrated that AHs are bioavailable to English sole living in polluted sediments and that these compounds form metabolites that are excreted into bile. For example, the concentrations of metabolites with BaP-like fluorescence (as shown by high-pressure liquid chromatography/fluorescence detection) were substantially greater in bile of fish from the Duwamish Waterway compared to bile of fish from nonurban sites, e.g., Port Madison (Krahn *et al.,* 1984). Several individual metabolites of AHs (e.g., 9,10-anthraquinone, hydroxyfluorene and anthracene carboxyaldehyde) were identified by gas chromatography/mass spectrometry in extracts of hydrolyzed bile of English sole from polluted waterways. Oxidations of the benzene rings of the aromatic nitrogen compounds to form phenols, diols and other metabolites are to be expected (Perin *et al.,* 1981), as indicated by studies of BaP metabolism in English sole (Gmur & Varanasi, 1982). In fact, a higher proportion of BaP 7,8-diol (the penultimate carcinogen of BaP) is formed in English sole liver compared to rat liver (Gmur & Varanasi, 1982).

In laboratory studies, we have also shown (Varanasi & Gmur, 1981a, b; Stein *et al.,* 1984) that sediment-associated AHs are readily bioavailable to English sole *via* non-dietary routes. Moreover, the ability of fish to metabolize xenobiotics has a significant influence on bioconcentration. For example, when English sole were exposed simultaneously to naphthalene (NPH) and BaP in sediment, the bioconcentration factor for NPH was considerably higher than that for BaP (Varanasi & Gmur, 1981a). Thus, even though both AHs were metabolized by English sole, BaP was metabolized to a much greater extent. However, in addition to the influence of metabolism on the bioconcentration of organic compounds, the ability to excrete the parent structures is also significant. In this regard, we know that low molecular weight AHs, such as NPH, are readily excreted via the gills and skin (Varanasi *et al.,* 1978; Thomas & Rice, 1982).

Stein *et al.,* (1984) also demonstrated that when English sole were exposed simultaneously to ^{3}H-BaP sediment and Aroclor 1254 (^{14}C-PCBs) in sediment, both BaP and PCBs were taken up. Notably, however, the BaP was extensively metabolized in comparison to the PCBs. Moreover, the rate of excretion of BaP metabolites was similar to the rate of uptake of BaP, whereas the rate of excretion of PCBs or their metabolites was considerably less than the rate of uptake of PCBs. Thus, the PCB body burdens in English sole progressively increased, whereas the body burdens of BaP remained essentially unchanged. The bile of sole contained high concentrations of BaP metabolites compared to PCB metabolites. This indicates that while the concentrations of AHs in sole from some contaminated areas may be low, AHs are continually being taken up and metabolized at a high rate. Also, many of these AHs are metabolized into oxidized compounds which are known carcinogens.

Additional evidence for relationships between AHs and disease in Puget Sound

Recently conducted field studies in Puget Sound have provided additional supportive evidence for a relationship between AHs and liver disease in English sole. In studies of metabolites of AHs, English sole with liver lesions (including neoplasia) had significantly higher bile concentrations of metabolites with BaP-like fluorescence than did fish without liver lesions (Krahn *et al.*, 1984).

In Eagle Harbor, high concentrations of the creosote hydrocarbons are present in the sediments of a major portion of the harbor (Table 2). Chlorinated organic compounds, including PCB's were found only in trace amounts. In fact, a number of the sites examined contained concentrations of AHs far exceeding those in Seattle's highly polluted Duwamish River (Malins *et al.*, 1984). Gross and histopathologic examinations of English sole from Eagle Harbor were performed and the prevalences of liver tumors and other liver abnormalities were determined. The findings indicated that a large portion of the English sole population is afflicted with liver tumors (30.8%, 16 of 52 fish) and degenerative diseases of the liver (84.6%, 44 of 52 fish), diseases which have been linked to toxic chemicals in other areas of Puget Sound (Malins *et al.*, 1984).

Conclusions and implications

Statistically significant correlations between chemicals in sediment and hepatic neoplasms in the bottom-dwelling fish are suggestive of a general cause-and-effect relationship. However, not all sediment-associated chemicals can be presently identified, thus unidentified compounds cannot be ruled out as principal etiological factors in the liver neoplasia (Malins *et al.*, 1984). For example, we recently investigated relationships between hepatic lesions in English sole and nitrogen heterocycles, a class of compounds which are not usually measured in chemical analyses of contaminated sediments. Using electron paramagnetic resonance (EPR) spectroscopy, we demonstrated that the concentrations of free radicals (N-oxyl derivatives of carbazoles) were higher ($p<0.05$) in microsomes from sole livers with lesions (including hepatic neoplasms) compared to microsomes from livers without lesions (Malins *et al.*, 1983a). Nitrogen-containing compounds are also readily metabolized by marine fish. For example, using electron paramagnetic resonance spectroscopy, English sole from polluted areas (i.e. the Duwamish River) were shown to contain N-oxyl free radicals in the liver which were derived from carbazoles (Malins *et al.*, 1983b; Roubal & Malins, 1985). Previous studies have identified a variety of nitrogen-containing compounds in polluted Puget Sound sediments, including a variety of carbazoles (Malins *et al.*, 1982).

It is clear that organic chemicals in sediments are readily transferred to bottom fish. Little is known, however, about the transfer of many such compounds from contaminated bottom fish to predators, including the human consumer. Substantial metabolism of AHs in fish obviously reduces the potential for food-chain transfer (Malins *et al.*, 1985a). In contrast, the refractory compounds (e.g., PCBs and HCB) appear to have a relatively high potential for being transferred through food chains to higher forms of life. For example, concentrations of PCBs and hexachlorobenzene were consistently higher in livers of English sole than in sediments from which they were taken. Concentrations of PCBs were also higher in organisms from the stomachs of English sole than in the sediments (Malins *et al.*, 1985b).

Interestingly, metal concentrations were not usually elevated in tissues of bottom fish from urban compared to nonurban marine environments of Puget Sound. For example, cadmium in the muscle ranged from not detected (< 10 ppb wet wt) to 20 ppb; lead ranged from not detected (< 20 ppb wet wt) to 250 ppb, with similar values for both urban and nonurban areas (Malins *et al.*, 1984). Our results which suggest that metals are not appreciably bioaccumulated in marine fish are consistent with results from other studies of coastal areas (Young, 1982; Sherwood, 1982).

The occurrences of liver neoplasia in the Puget Sound fish are not isolated examples. As indicated (Baumann *et al.,* 1982; Black *et al.,* 1982; Black, 1983), high prevalences of neoplastic lesions have been found in bottom fish from a number of other areas where there are chemically-contaminated sediments. Even though there are clues, there is little firm evidence about the actual causes of these neoplasms and further insight into their etiology will require more study, particularly in the laboratory. Moreover, polluted environments that are conducive to the induction of fish neoplasia may also be responsible for a host of other serious and presently unrecognized changes at both the organismal and ecosystem levels.

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