Relating Field and Laboratory Studies: Cause-and-Effect Research

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Field surveys have played, and will no doubt continue to play, a critical role in assessing the health of Puget Sound. One major component of such field surveys is the measurement of concentrations of the myriad sediment-associated chemical contaminants often found in the Sound's urban embayments. Another major component of most field surveys focuses on direct and indirect measurement of biological effects. Among the more commonly used measures of effects have been determination of the prevalences of a variety of liver lesions in bottom-dwelling marine fishes (*e.g.* English sole), and assessment of sediment toxicity in bioassays using selected life stages of a variety of invertebrate species .

The theme of the present scientific session is the question of how good are biological effects measures as indicators of pollution-associated problems. One of the critical factors in addressing this question is knowledge of how strong is the evidence linking the effect (*e.g.* prevalence of neoplasms, significant toxicity in a sediment bioassay) to its cause(s). Unfortunately, available data on cause-and-effect relationships between many biological effects and environmental causes are, at best, relatively limited. The bright side is, however, that this situation is gradually changing. In our laboratories the findings from several recently completed studies are yielding data on cause-and-effect relationships that should be of considerable value to environmental managers in interpreting the significance of the findings of biomonitoring programs.

The use of histopathology to monitor prevalences of liver diseases in bottom-dwelling species has been used in field surveys in Puget Sound for over a decade (see Myers *et al.*, this volume). Indeed, it was the growing body of evidence

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from these studies that first suggested the relationship between liver tumors and exposure to sediment-associated contaminants (Malins *et al.*, 1984). Placing this relationship on an even more solid basis has been recent work in our laboratories using the methods of analytical epidemiology to further refine and strengthen the argument for this relationship. Using data from six field surveys conducted by our research group between 1979 and 1985, a model relating prevalences of hepatic neoplasms in English sole to fish size and sediment concentrations of selected classes of xenobiotics has been constructed. The proposed model (Figure 1), which is based on 66 collections of fish at 46 sampling sites in Puget Sound, accounts for approximately 35% of the observed variation in prevalences of hepatic neoplasms in English sole. Fish size exerted the greatest positive effect (15.3%), with observed neoplasm prevalences being greater when only large fish were selected than when the entire size range was employed. Moreover, neoplasm prevalences were positively correlated with sediment concentrations of both aromatic hydrocarbons (AHs, 12.1%) and with polychlorinated biphenyls (PCBs, 1.0%), and negatively correlated with sediment concentrations of chlorinated butadienes (2.4%). Interestingly, neoplasm prevalences were also negatively correlated with the interaction of AHs and PCBs (4.5%), suggesting a modulating effect of one or both of these classes of xenobiotics on the carcinogenicity of these compounds.

Notwithstanding the value of this model in contributing to a greater understanding of the etiology of contaminant related liver neoplasia in English sole, it is not *de facto* evidence for a cause-and-effect relationship. We are, however, addressing this issue in a series of controlled laboratory studies in which the tumorigenicity of organic-solvent extracts of urban sediments are being tested in this species. The results of this study, which will be reported in detail elsewhere (Schiewe *et al.*, manuscript in preparation), revealed a significantly higher ($p \leq 0.05$) incidence of a spectrum of lesions, including nuclear pleomorphism, megalocytic hepatosis, and preneoplastic foci of cellular alteration, in English sole exposed to a fraction of an extract of Eagle Harbor sediment enriched for high molecular weight AHs and nitrogen-containing aromatic compounds (Figure 2). English sole exposed to the model hepatocarcinogen benzo[a]pyrene (BaP) also exhibited elevated prevalences of these same lesions. Control groups of English sole, including the fish exposed to a similarly-prepared extract of West Beach sediment (a relatively uncontaminated reference site in northern Puget Sound), fish exposed to the carrier only, or fish that were not exposed, did not develop these same lesions. Moreover, lesions produced in the Eagle Harbor sediment extract-exposed sole were indistinguishable from those routinely

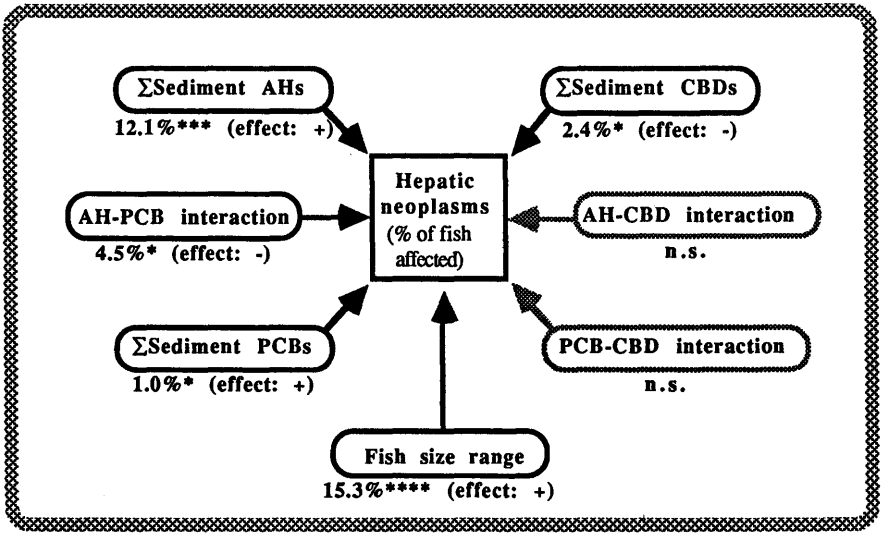

FIGURE 1. Model summarizing logistic regression analyses relating sediment concentrations of aromatic hydrocarbons (AHs), polychlorinated biphenyls (PCBs) and chlorinated butadienes (CBDs) to prevalences of liver neoplasms in English sole from Puget Sound. The model (AHs + PCBs + fish size - CBDs- [AH + PCB interaction] accounts for 35.3% of observed variation in neoplasm prevalence (% = percentage variation in neoplasm prevalence explained; n.s. = effect not significant [$p > 0.05$]; * = effect significant [$p \leq 0.01$]; ** = effect significant [$p \leq 0.001$]; *** = effect significant [$p \leq 0.00001$]. An earlier version of this model has been previously published (Malins et al., 1988).

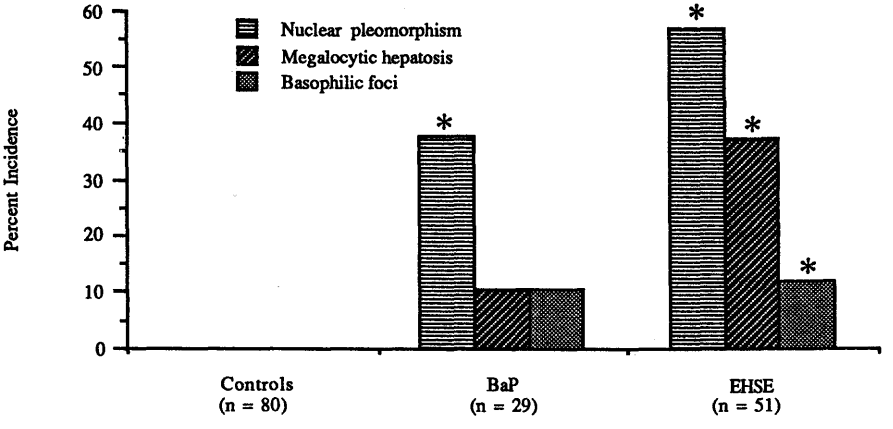

FIGURE 2. Incidence (%) of selected hepatic lesions in English sole (Parophrys vetulus) parenterally exposed to Eagle Harbor sediment extract (EHSE) or benzo[a]pyrene (BaP) for one year. (* indicates an incidence significantly different from control [$P \leq 0.05$]).

observed in English sole captured in this creosote-contaminated embayment (Malins *et al.*, 1985; Myers *et al.*, 1987). Hence, these data directly implicate the sediment contaminants as the cause of the lesions and speak to the scientific soundness of using histopathology as a monitoring tool for adverse effects of chemical contaminants.

Bioassays have a long history of use in aquatic toxicology and in the past decade their use to assess sediment toxicity in field surveys in Puget Sound has become commonplace. Sediment bioassays also are used extensively in the evaluation of dredged sediments to evaluate disposal options. One of the more commonly employed sediment bioassays in the Puget Sound basin has been one measuring 10-day survival of the infaunal amphipod, *Rhepoxynius abronius*. Despite its widespread use, little was known until recently about the bioavailability of most of the more commonly encountered chemicals in sediments to, and their potential effects on, this species. In addressing the need for information about this species, we have been conducting a series of studies with *R. abronius* and sediments supplemented with defined mixtures of chlorinated and aromatic hydrocarbons. (Plesha *et al.*, Mar. Environ. Res., in press). These studies were designed to build on results of previous studies in our laboratories that demonstrated the bioavailabilty of a variety of 3 to 5 ring AHs from contaminated sediment to *R. abronius* as well as the capability of this species to metabolize and excrete these compounds (Varanasi, *et al.*, 1985).

For the amphipod investigations, a reference sediment from Hood Canal was supplemented with mixtures of 7 AHs or 4 chlorinated hydrocarbons (CHs). The compounds selected were all considered contaminants of concern based on their toxicity to selected marine species and their presence and persistence in urban areas of Puget Sound (Konasewich *et al.*, 1982). The nominal concentrations of the individual components were selected to approximate the highest concentration of each compound measured in sediment from either the Hylebos or Duwamish Waterways of Puget Sound (Malins *et al.*, 1982).

Results of the 10-day bioassays of the AH- and CH- amended sediments are summarized in Table 1. Significantly higher mortality, compared to controls, occurred among amphipods exposed to the CH-amended sediments at both the 5X level and at the level approximating that measured in contaminated sediments of Puget Sound. In contrast, significantly higher toxicity was observed with the AH-amended sediment only at the 5X level. Also assessed as part of these experiments was the degree of exposure of the amphipods to AHs and CHs based on uptake of representative radiolabeled compounds (*i.e.* naphthalene, BaP, PCBs).

TABLE 1. Survival of amphipods (*Rhepoxynius abronius*) following a 10-day exposure to sediments supplemented with mixtures of aromatic or chlorinated hydrocarbons. (Data from Plesha *et al.*, Mar. Environ. Res., in press).

Sediment	Percent Survival ($\overline{X}$ ± s.d.)
Control (Untreated)	94.0 ± 5.5
Solvent Control (Acetone treated)	94.0 ± 4.2
1x Aromatic Hydrocarbons	95.0 ± 6.2
5x Aromatic Hydrocarbons	83.0[a] ± 2.8
1x Chlorinated Hydrocarbons	69.0[a] ± 7.4
5x Chlorinated Hydrocarbons	15.0[a] ± 13.7

[a] Significantly different from control (P = 0.05).

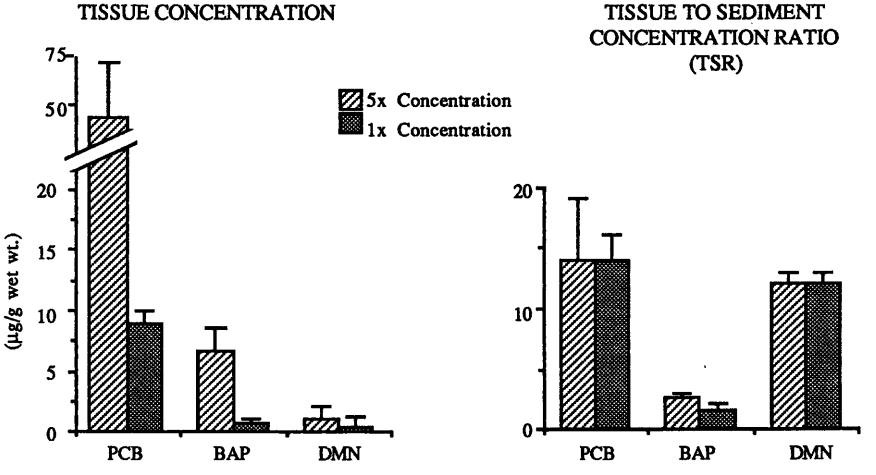

FIGURE 3 Concentrations of radiolabeled polychlorinated biphenyls (PCBs), benzo[a]pyrene (BaP)and 2,6-dimethylnapthalene (DMN) in the amphipod, Rhepoxynius abronius, and tissue to sediment ratios (TSRs) following a 10-day exposure to aromatic and chlorinated hydro-carbon-amended sediments. (Data from Plesha et al., Mar. Environ. Res., in press).

Measurement of radioactivity incorporated by *R. abronius* after the 10-day exposure period indicated a dose-related uptake of xenobiotic compounds from both the CH- and AH-supplemented sediments (Figure 3). While it is clearly a step forward to demonstrate a dose-related response to sediment concentrations of xenobiotics, it is an even greater step to relate sediment concentrations of xenobiotics to uptake and uptake, in turn, to effect. In these studies the latter was indeed the case. Although these data on uptake and toxicity of sediment-sorbed contaminants support the use *R. abronius* to assess sediment quality, there are several additional questions regarding, for example, influence of physical factors (*e.g.* grain size distribution; sediment redox potential) that are yet to be addressed. Clearly, a thorough understanding of all factors -- physical, chemical and biological -- that effect survival of a bioassay test species, is required for intelligent use of a bioassay in biomonitoring.

Although the list of sediment bioassays available to the environmental manager is gradually expanding, the majority of such bioassays focus on acute lethal effects, and to a lesser extent on acute sublethal effects. There are few sediment bioassays that focus on the potential of sediment to produce long-term or chronic effect. Hence there is a critical need to concentrate greater research effort in this area. In this regard, recent work in our laboratories include investigations exploring the feasibility of using juvenile sand dollars (*Dendraster excentricus*) and an aquarium fish, the Japanese medaka (*Oryzias latipes*) in chronic effects bioassays. This emphasis on chronic effects does not, however, obfuscate the need to continue examining cause-and-effect relationships with routinely used bioassay test species. For example, just as we have investigated the uptake and toxicity of selected sediment-sorbed contaminants in *R. abronius*, there is a need to conduct similar investigations with larvae of the Pacific oyster (*Crassostrea gigas*), a species and life-stage widely employed in sediment bioassays.

In summary, we have presented an overview of the results of two recently completed laboratory studies that provide insight into the utility of two of the more commonly used bioindicators in Puget Sound field surveys. In the case of histopathological monitoring of fish lesion prevalence, laboratory studies support a cause-and-effect relationship between selected liver lesions, including presumptive preneoplastic lesions, and exposure to sediment-associated organic contaminants, particularly aromatic compounds. In amphipod bioassays with *R. abronius*, these data demonstrate dose related uptake and toxicity of sediment-sorbed aromatic and chlorinated hydrocarbons. Such insight into cause-and-effect relationships clearly enhances the ability

to more fully understand and interpret the significance of findings from intensive field surveys.

Acknowledgements

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