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Disposition of Xenobiotic Chemicals and Metabolites in Marine Organisms

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Studies with several bottom fish species from urban waterways show that of the identified xenobiotic chemicals in bottom sediments, polycylic aromatic hydrocarbons (PAHs) are the most strongly associated with the prevalence of liver lesions, including neoplasms. Accordingly, there is concern about the transfer of contaminants, such as PAHs, from aquatic species to humans. Because PAHs exert their toxicity only after being biotransformed, increasing attention has been focused on the ability of aquatic organisms to metabolize these chemicals. Overall, the results of both laboratory and field studies show that generally low levels (nanograms per gram wet weight) of a few low molecular weight PAHs may be present in edible tissue of fish from contaminated areas and that high molecular weight PAHs, such as the carcinogen benzo(*a*)pyrene, will rarely be detected because of extensive metabolism. Additionally, the results from a few studies suggest that even though interactions between xenobiotics can affect both biochemical and physiological systems to alter the disposition of PAHs in fish, these interactions do not markedly change the relative proportions of metabolites to parent PAH in tissues. Thus, these studies clearly demonstrate that to obtain some insight into the questions of whether there is any risk to human health from consuming fish and crustaceans from urban areas, techniques must be developed that measure metabolites of carcinogens, such as PAHs, in edible tissue. Initial attempts may focus on semiquantitative methods that permit rapid assessment of the level of metabolites in edible tissues of fish and crustaceans from many urban areas. Based on information from such screening studies, further refinement in methodology leading to identification of specific compounds may be needed because certain metabolites may not be as toxic or carcinogenic as others. A scientifically sound database on a broad spectrum of contaminants and their metabolites in aquatic species is needed before we can adequately address the question of bioavailability or food chain transfer of these compounds to humans.

Introduction

Reports of high prevalences of liver neoplasms in bottom fish from urban waterways have heightened our awareness of the potential problems concerning the quality of fisheries habitats, as well as of food-chain transfer of contaminants from fish and shellfish to humans. In a separate paper included in this issue, Myers et al. (*1*) have discussed detailed evidence gained from field and laboratory studies showing consistent associations between the presence of certain chemical contaminants and the prevalence of neoplasms in a commercially and recreationally important fish species, English sole (*Parophrys vetulus*). These studies have systematically demonstrated that certain liver lesions, including preneoplastic lesions and probably neoplasms, in English sole are caused by exposure to environmental pollutants. Although similar detailed information demonstrating cause-and-effect relationships between contaminant exposure and liver lesions in other wild fish species (e.g., white croaker, *Genyonemus lineatus*), and winter flounder (*Pseudopleuronectes americanus*) is not available, there are sufficient epizootological data to suggest that certain lesions, including neoplasms, in these fish species are also pollution-induced (*2,3*).

The studies with English sole in Puget Sound show that of the identified organic xenobiotic chemicals in bottom sediments, polycyclic aromatic hydrocarbons (PAHs) were the most strongly associated with the prevalence of liver lesions; however, a weaker correlation was also suggested for certain chlorinated compounds (*4*). Because carcinogenic compounds such as PAHs exert their toxicity only after being biotransformed, increasing attention has been focused on the ability of aquatic organisms to metabolize these chemical carcinogens that are present in urban waterways. As the emphasis of this conference was on carcinogens in aquatic food species, we will limit our discussion in this paper to the processes and mechanisms that govern the distribution of PAHs and their metabolites in fish. Data from field studies on levels of PAHs in fish from polluted sites will be discussed only to illustrate the importance of metabolic pathways in the toxicokinetics of PAHs in these organisms.

A recent review of the literature on the metabolism and disposition of PAHs in fish indicates that carcinogenic PAHs such as benzo(*a*)pyrene (BaP) are extensively metabolized by most fish species (*5*). The noteworthy finding of recent studies conducted with radiolabeled hydrocarbons is that the highest concentrations of radioactivity, which are primarily due to PAH metabolites, are found in the hepatobiliary system of fishes, with edible muscle and other extrahepatic tissues having rather low concentrations (*6*). The relative ranking of concentrations, as shown in Figure 1, is generally bile $\gg$ liver $>$ skin $>$ muscle regardless of the species, route (sediment, water *per os*), or mode (continuous versus single dosage) of exposure.

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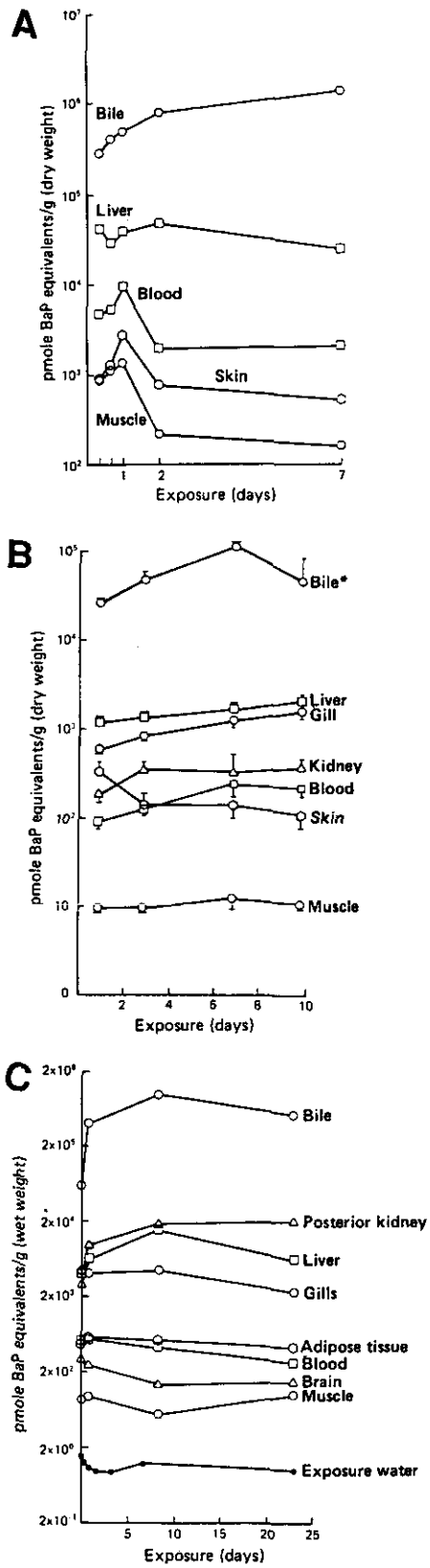

FIGURE 1. Disposition of radioactivity in tissues of fish. (*A,B*) English sole (*Parophrys vetulus*); (*C*) Northern pike (*Esox lucius*) exposed to ^{3}H-BaP via (*A*) diet (2 mg/kg body weight), (*B*) sediment (3 μg/g sediment, dry weight), and (*C*) water (75 ng BaP/L). Adapted from Varanasi et al. (*5*).

Results from Field Studies on Levels of PAHs in Edible Tissue

The findings from laboratory studies on disposition of aromatic hydrocarbons in fish are corroborated by the results from several field studies showing that concentrations of potentially carcinogenic, high molecular weight PAHs (3–5 benzenoid rings) in muscle tissue of bottom fish sampled from urban areas are typically near or below the limits of detection. For example, most of the PAHs recently found (*7*) at detectable levels in muscle of English sole from contaminated sites in Puget Sound, Washington, were typically of the noncarcinogenic, low molecular weight PAHs, including naphthalene, 1- and 2-methylnaphthalene, biphenyl, acenapthene, and 2,6-dimethylnaphthalene, which are not as effectively metabolized as higher molecular weight PAHs (*8*). The naphthalenes were detected at higher concentrations in muscle of fish from a creosote-contaminated site, Eagle Harbor, Washington, than in muscle of fish from relatively uncontaminated sites (Table 1); whereas the high molecular weight PAHs, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*e*)pyrene, and perylene, were detected at low levels in fish from these sites. The known carcinogen BaP was not detected in the muscle of any fish sampled in this study.

Landolt et al. (*9*) recently assessed the levels of PAHs (Table 1) in edible muscle of several species of fish that are of importance to the recreational sport fishery in Puget Sound. They reported that levels of PAHs ranged from trace levels (< 1 ppb) to 32 ppb, wet weight, when measured by HPLC fluorescence. However, most of these levels could not be confirmed by GC/MS. In a related study specifically examining the potential human health risk from consumption of bottomfish from Commencement Bay, Washington, low levels of only a few aromatic hydrocarbons were detected in muscle of bottomfish. It was concluded from this risk assessment study that the levels of aromatic hydrocarbons in muscle of bottom fish from Commencement Bay were not sufficiently high to pose a threat to human health, whether the assessment was for carcinogenic or acute/chronic noncarcinogenic risk (*10*).

Overall, the results of these studies suggest that, generally, low levels of a few low molecular weight aromatic hydrocarbons, such as naphthalenes, could be present in edible muscle of fish from urban areas and that high molecular weight PAHs, such as BaP, will rarely be detected, because of extensive metabolism. However, certain exceptions to this pattern of distribution may occur. For instance, the lowering of hepatic cytochrome P-450 levels in sexually mature fish (*11*) may alter the organism's ability to metabolize and excrete PAHs, which in turn may result in altered disposition of PAHs and metabolites into extrahepatic tissues (*11–13*). In cases of acute exposure to hydrocarbons, such as in an oil spill, the ability of an organism to process PAHs may be altered by the presence of polar components, including high concentrations of PAH metabolites (*14*) produced during the metabolism of petroleum-derived aromatic hydrocarbons. Furthermore, exposure to other xenobiotic chemicals, which may induce [e.g., polychlorinated biphenyls (PCBs)] or inhibit (e.g., phenols and specific PCB congeners) xenobiotic-metabolizing enzyme systems (*11,15*) or alter excretory pathways (*16*), can have

Table 1. Range in concentrations of summed PAHs in muscle tissue of English sole from Puget Sound.[a]

Site	Summed low molecular weight PAHs	Specific low molecular weight PAHs detected	Summed high molecular weight PAHs	Specific high molecular weight PAHs detected
Eagle Harbor	71-103[b]	Naphthalene, 1- and 2-methylnaphthalene, biphenyl, 2,6-dimethylnaphthalene and acenaphthene	38-39	Fluorene, phenanthrene fluoranthene, perylene, and benzo(*e*)pyrene
Duwamish Waterway	8.8-26	Naphthalene, 1- and 2- methylnaphthalene	13-21	Fluorene, phenanthrene, and anthracene, pyrene
Elliot Bay (Denny way)	9.2-14	Naphthalene, 1- and 2- methylphthalene	5.0-7.1	Phenanthrene and anthracene
Everett Harbor	8.6-13	Naphthalene, 1- and 2-methylnaphthalene	4.6- 5.7	Phenanathrene, anthracene
Commencement Bay (Hylebos)	0-15	Naphthalene, 1- and 2-methylnaphthalene	0-3.4	Phenanthrene
Sinclair Inlet	13-15	Naphthalene, 1- and 2-methylnaphthalene	0-2.5	Phenanthrene
Case Inlet (reference)	0-12	Naphthalene, 1- and 2-methylnaphtnalene	2.3-9.4	Phenanthrene

[a]Adapted from Varanasi et al. (*7*). PAHs, polyaromatic hydrocarbons.
[b]Values are nanogram/gram wet weight muscle.

significant effects on the ability of fish to process carcinogens and potentially could affect the pattern of distribution of carcinogenic compounds and their metabolites in fish, including the levels in edible flesh.

Environmental and Physiological Factors Affecting PAH Disposition

The effects of environmental contaminants on the disposition of PAHs have received relatively little attention. The studies to date have concentrated mainly on the effects of mixed-function oxidase (MFO) inducers on tissue and fluid levels of a number of PAHs, but in particular naphthalene and its alkylated derivatives. For example, the exposure of salmonids to MFO inducers [e.g., benz(g)anthracene (BaA), β-naphthoflavone, and PCBs] followed by exposure to radiolabeled naphthalene or an alkylated naphthalene results in consistently increased levels of radioactivity in bile of induced fish compared to controls, suggesting enhanced excretion of PAHs (*17-19*). Additionally, in two studies, English sole were exposed to BaP and PCBs added to sediment either singly or together or to sediments from a contaminated site and a reference (relatively clean) site, both containing trace levels of ^{3}H-BaP (*6,20*). The results of both studies showed significantly greater accumulation of BaP and its metabolites in liver and bile of English sole exposed to BaP in the presence of other xenobiotics relative to that observed in fish exposed exposed to BaP alone (Fig. 2) (*6,20*). The cumulative results from these studies on the effects of exposure to inducers of cytochrome P-450 on PAH metabolism *in vivo* are that the concentrations of PAH metabolites in bile of fish are increased and that the effect on levels in liver, and presumably other tissues, is unpredictable. The differences among studies as to the effect of P-450 inducers on the disposition of PAHs in liver may be due to a number of factors, which include the route of exposure, specific inducer used, and structure of the PAH whose disposition is being examined. Further, it has been demonstrated that the rate of excretion of PAH metabolites can be inhibited by other substrates of secretory transport systems. For example, the renal excretion of BaP-7,8-diol conjugates by southern flounder (*Paralichthys lethostigma*) was shown to be markedly retarded by pretreatment with the herbicide 2,4-dichlorophenoxyacetic acid (*16*).

Overall, these studies serve to demonstrate that interactions between xenobiotic chemicals can affect both biochemical and physiological systems to alter the disposition of PAHs in fish. Detailed information on the interactive effects of xenobiotics on pathways of absorption, metabolism, and excretion of PAHs is necessary to accurately predict effects of co-exposure to xenobiotics on the toxicokinetics of PAHs and thus levels of PAHs in edible flesh. Nevertheless, the data on PAHs in edible tissue of fish from urban areas supports the assumption that in most cases unmetabolized carcinogenic PAHs may not be detected or detected at only low parts per billion levels even in fish from highly contaminated areas.

In addition to the potential of other contaminants to affect the metabolism and disposition of PAHs in fish, species-specific differences in biochemical and physiological parameters, such as basal levels of xenobiotic-metabolizing enzymes and lipid content of tissues, appear to have significant effects on the disposition of PAHs and their metabolites. Broad-based comparisons between species is difficult because no single PAH has been used universally in studies with fish; however, Solbakken and coworkers have exposed several species of fish to [^{14}C] phenanthrene (PHN) intragastrically and measured levels of PHN-derived radioactivity in tissues and fluids at common sampling times (Table 2) (*21-23*). In the teleosts, rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), and coalfish (*Pollachius virens*), the gall bladder contained >3% of the dose at 24 hr postexposure, whereas in spiny dogfish (*Squalus acanthias*), an elasmobranch, the gall bladder contained only 0.1% of the dose at 24-hr sampling time. The difference in the accumulation of PHN metabolites between teleosts and elasmobranchs is apparently related to the low hepatic MFO activities in spiny dogfish compared to teleosts (*24*). Moreover, the low level of accumulation of PHN metabolites in bile of spiny dogfish is not specific to PHN. Similar studies have shown high accumulation of naphthalene in liver (22% of the dose) and only small amounts in gall bladder (0.03 % dose) of spiny dogfish (*25*).

The influence of lipid content of organs on accumulation of PHN-derived radioactivity also explains, in part, the differences in the results shown in Table 2. The coalfish is a lean fish having major lipid reserves in liver, whereas the rainbow trout is a fatty fish having lipid distributed throughout the body. Correspondingly, the ratio of the percent dose of PHN in liver to that in muscle is markedly greater for coalfish, 12, than for trout, 0.28. Further, the grunt (*Haemiilon sciurus*) and spiny dogfish have higher hepatic lipid contents (20% to 50%, respectively) than

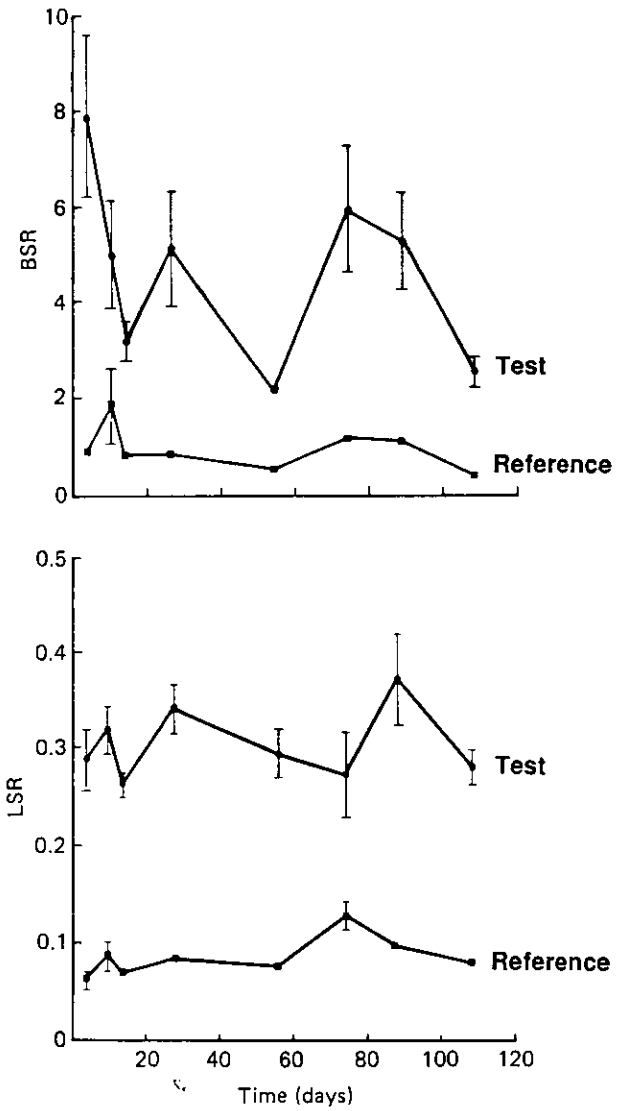

FIGURE 2. The ratios of the concentration of BaP-derived radioactivity in bile and liver to that in sediment for English sole exposed to contaminated (test) and reference sediments for up to 108 days. BSR (pmole BaP equivalents/g bile wet weight)/(pmole BaP/g sediment we weight; LSR (pmole/BaP equivalents/g liver wet weight)/(pmole BaP/g sediment wet weight) The sediments, collected from a contaminated (Duwamish Waterway) and a reference (Dosewallips) site in Puget Sound, Washington, were spiked with trace levels of ^{3}H-BaP. Data are shown as $\bar{X} \pm$ SEM (n = 6 to 16). (*) Indicates significant effect due to exposure of sole to the contaminated sediment relative to exposure to the reference sediment. Reprinted from Stein et al. (*20*).

Table 2. Percent administered dose of phenanthrene in muscle, liver, and bile of fish.[a]

	Rainbow trout[b]	Coalfish	Grunt	Spiny dogfish
Muscle	6.4	3.9	3.5	3.3
Liver	1.8	47	22	21
Bile	4.9	3.3	NR[c]	0.1

[a]Adapted from Solbakken et al. (*21–23*).
[b]Fish were exposed to ^{14}C-phenanthrene intragastrically and tissues were sampled 24 hr after exposure.
[c]NR, not reported.

trout (10%) and also accumulate high levels of PHN in liver compared to muscle. Additionally, in the lean fish, English sole, exposed to sediment-associated BaP or to BaP intragastrically, liver and bile had the highest concentrations of BaP and its metabolites (*6,20*). It is not surprising that the hepatobiliary system accumulates high levels of PAHs and their metabolites, because liver is the tissue that usually has the highest levels of xenobiotic-metabolizing enzyme activities (*11*); however, the relative lipid contents of liver and muscle of fish also appear to play an important role in determining the relative disposition of PAHs and metabolites in edible flesh.

PAH Metabolites in Edible Tissue

As discussed above, the laboratory studies of PAH metabolism and disposition show that tissues and fluids of fish exposed to radiolabeled PAHs primarily contain metabolites. Moreover, the ratio of metabolites to parent PAH in tissues generally increases with time, showing relative persistence of PAH metabolites in fish tissues (*5*). These findings do not appear to be PAH dependent because similar findings have been obtained for fish exposed to naphthalene, PHN, or BaP, three PAHs that have markedly different molecular weights and show differences in their extent of metabolism by fish (*5,8*). Furthermore, based on a relatively limited number of studies (*26*), it would appear that many crustaceans also metabolize PAHs effectively, and the hepatopancreas of these organisms accumulate high concentrations of metabolites.

At present, there are no quantitative methods similar to those available for parent PAHs for analysis of metabolites in tissues of fish. Krahn et al. (*27,28*) developed a semiquantitative method that has allowed an estimation of the level of metabolites of fluorescent aromatic compounds (FACs) in bile of fish from field studies. Some of the FACs detected in bile of fish from a PAH-contaminated site have been identified as PAH metabolites (Fig. 3) (*29*). Findings from the field studies showed high levels of FACs in bile of fish from urban areas and thus demonstrated the bioavailability of PAHs and also provided much needed supportive evidence for the strong correlation between levels of sediment PAHs and prevalence of liver lesions in bottomfish from several contaminated sites (*1*).

Clearly, then, to obtain some insights into the question of whether there is any risk to human health from consuming fish from urban areas, techniques must be developed that measure metabolites of carcinogens in edible tissues of fish and shellfish. A rapid method comparable to that for measuring FACs in bile would be an appropriate first step to screen edible flesh of fish from urban areas for the presence of metabolites of carcinogenic PAHs. It must be emphasized, however, that assessing the level of FACs in bile or tissue is only a semiquantitative assessment of contaminant exposure, and for any method to have value in human health risk assessment, it must be able to identify and quantify individual metabolites in edible flesh. Considering the fact that only certain metabolites of higher molecular weight PAHs, such as BaP, are known proximate carcinogens, it is imperative to develop more sensitive and reliable methods for detection of individual metabolites in edible flesh of fish and crustaceans.

The use of radiolabeled PAHs has demonstrated that the very low levels of radioactivity detected in muscle of PAH-exposed

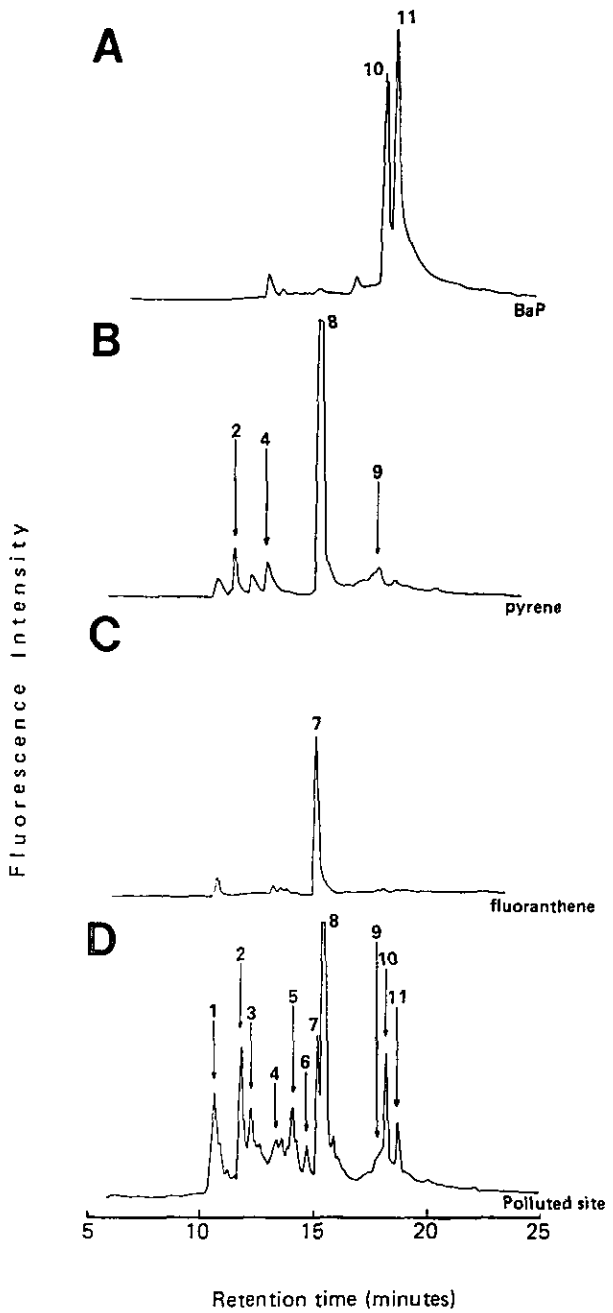

FIGURE 3. HPLC/fluorescence chromatograms of bile from English sole injected with (*A*) BaP, (*B*) pyrene, (*C*) fluoranthene, and of bile from (*D*) an English sole, from a polluted site (Eagle Harbor) in Puget Sound. Most of the peaks in the English sole from the contaminated site were metabolites of PAHs, as indicated by the numbers, and were further identified by GA/MS and stop-floe HPLC flourescence spectroscopy. From Krahn et al. (*29*).

fish is primarily composed of metabolites (Table 3). Accordingly, analysis of fish muscle and probably edible tissue from most crustaceans for parent PAHs would yield very little useful information. To date, attempts to analyze PAH metabolites in fish muscle have not been successful, primarily because of their low levels and also because of the presence of interfering lipid and other biogenic compounds that hamper their extraction and quantitation. Nevertheless, it should be possible to extract PAH metabolites from fish muscle, and once extracted, their fluorescence at wavelengths specific for carcinogenic hydrocarbon metabolites can be measured to provide estimates of the presence of such compounds. Additionally, these extracted metabolites can be tested for genotoxicity using the Ames test or similar methodology.

The problem of extracting polar metabolites (e.g., conjugates) from tissues and not inducing substantial structural change may also be a formidable technical problem. However, one preliminary study (*7*) reports testing of muscle tissue extracts from English sole from several sites in Puget Sound for mutagenicity using the Ames test. The extracts were tested both before and after enzymatic (β-glucuronidase) treatment to hydrolyze glucuronide conjugates and thereby generate higher levels of primary metabolites, such as phenols and diols. In addition, the testing was conducted both with and without the addition of a microsomal enzyme preparation (S-9 from rats) intended to simulate metabolic activation of mutagens. The results of this preliminary study showed that only one β-glucuronidase-treated sample from a highly contaminated site elicited a mutagenic response. However, this extract did not produce a dose-related increase in colony counts. These results are only suggestive that such testing may be useful and illustrate that many technical difficulties need to be solved. Even with surmounting these difficulties, the results may always be equivocal because considerable manipulation of samples is necessary to obtain appropriate extracts.

The presence of genotoxic metabolites in edible tissue can also be deduced indirectly by measuring metabolites bound to DNA or protein. The level of metabolites bound to DNA or protein in edible tissue of organisms from urban areas may be miniscule; therefore, the most practical approach would be to use highly sensitive but nonspecific procedures that would detect metabolites from a broad spectrum of contaminants. The ^{32}P-postlabeling assay, used to measure xenobiotic metabolites bound to hepatic DNA in fish from urban areas, has a very low limit of detection for adducts of hydrophobic aromatic xenobiotics and does not require characterization of individual adducts before they can be measured. Moreover, this assay yields a fingerprint pattern of adducts, thereby giving some indication of the types of contaminants adducted to DNA (*30,31*). Furthermore, a preliminary study in our laboratory shows that this technique can be adapted to extrahepatic tissues of fish (unpublished results). Detection of specific carcinogenic metabolite-DNA adducts in edible tissue may also be useful to indicate

Table 3. Disposition of PCBs and BaP in muscle of English sole.

	Percent of total radioactivity		
	Sediments[a]		Intragastric[b]
Exposure	PCBs	BaP	BaP
Parent compounds	99	11	6
Metabolites	1	82	94[c]

[a]Tissue samples were from English sole exposed to a contaminated sediment from an urban area spiked with ^{14}C-PCBs and ^{3}H-BaP. Adapted from Stein et al. (*20*). PCBs, polychlorinated biphenyls; BaP, benzo(a)pyrene.

[b]English sole were exposed *per os* to 2 mg ^{14}C-BaP/kg body weight (*5*).

[c]Of the total metabolites, about 25% were primary metabolites (i .e., nonconjugates), and about 15% were glucuronide conjugates.

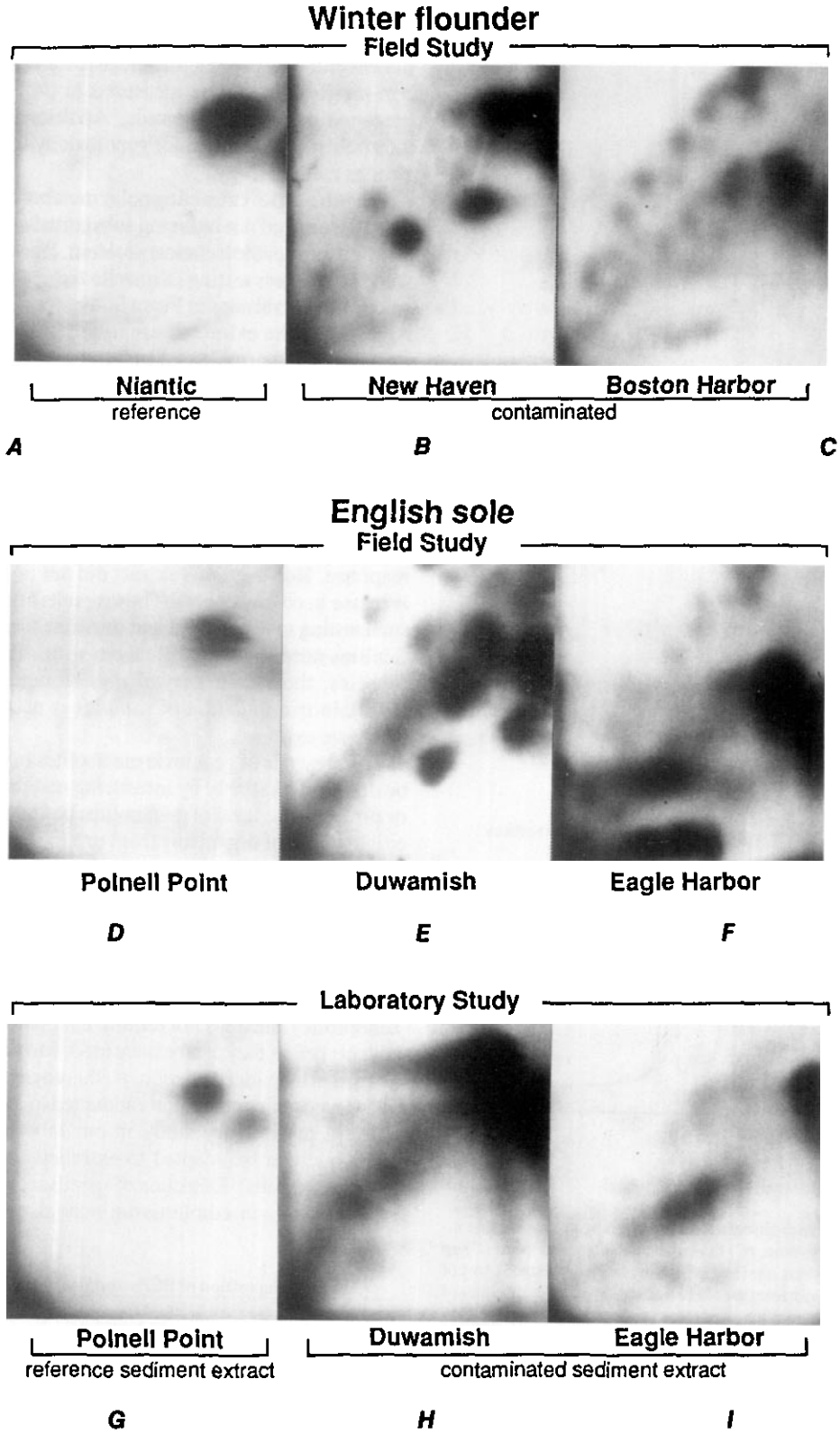

FIGURE 4. TLC profiles of ^{32}P-labeled DNA-xenobiotic adducts from benthic fish exposed to chemical contaminants. Panels *A–F* are autoradiograms of ^{32}P-labeled digests of hepatic DNA from winter flounder and English sole sampled from reference and contaminated sites, as shown. Panels *G–I* are autoradiograms of ^{32}P-labeled digests of hepatic DNA from reference English sole exposed to extracts of sediments from the reference site and the contaminated sites in Puget Sound, Washington. The winter flounder were sampled from a reference and contaminated site in Long Island Sound (Niantic and New Haven, respectively) and a contaminated area of Boston Harbor, Massachusetts. Adapted from Varanasi et al. (*30*) and Stein et al. (*31*).

contamination by PAHs. For example, measurement of fluorescence from BaP tetrols released on acid hydrolysis of DNA isolated from fish tissues may be used as a screening method for PAH-contamination (*32*).

The presence of genotoxic metabolites bound to cellular macromolecules in edible flesh may indicate the presence of precursor molecules in flesh at levels that are not easily detected by other analytical techniques. It is obvious that considerable effort needs to be directed toward development of sensitive and reliable techniques to measure levels of PAH metabolites in edible tissues of fish and shellfish. Our initial attempts are focused on semiquantitative methods that permit rapid assessment of the level of metabolites in edible flesh of fish and crustaceans from many urban areas. Based on information from such screening studies, further refinement in methodology leading to identification of specific compounds may be needed to properly address the issue of potential health risk of consuming such products.

It is apparent that detailed mechanistic studies evaluating metabolism and disposition of contaminants in aquatic species, using radiolabeled compounds, should continue as these studies provide valuable information for developing appropriate analytical strategies for testing environmental samples. For example, a study using radiolabeled PCBs showed that English sole metabolized PCBs (Aroclor 1254) but that liver accumulated primarily parent compounds. It was suggested that because PCBs were largely converted into glucuronide conjugates rather than thioether conjugates, the PCB metabolites from sole liver were rapidly excreted into gall bladder (*19*). In contrast, BaP is converted largely to thioether conjugates, which persist in tissues (*33*). It can be assumed, therefore, that metabolites of xenobiotics such as PCBs may not accumulate in edible flesh of fish as do the parent compounds. Thus, the issue of xenobiotic metabolites in edible tissues may not be equally important for all contaminants that are metabolized by fish because certain metabolites may not be as toxic or carcinogenic as others and also that certain metabolites may persist in tissues whereas others may be easily excreted into bile and hence removed. Hence, that a scientifically sound database on a broad spectrum of contaminants and metabolites in aquatic species is needed before we can adequately address the question of bioavailability or food chain transfer of these compounds to humans.

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