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USING REGIONAL EXPOSURE CRITERIA AND UPSTREAM REFERENCE DATA TO CHARACTERIZE SPATIAL AND TEMPORAL EXPOSURES TO CHEMICAL CONTAMINANTS

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Abstract—Analyses of biomarkers in fish were used to evaluate exposures among locations and across time. Two types of references were used for comparison, an upstream reference sample remote from known point sources and regional exposure criteria derived from a baseline of fish from reference sites throughout Ohio, USA. Liver, bile, and blood were sampled from white suckers (*Catostomus commersoni*) and common carp (*Cyprinus carpio*) collected during 1993 and 1996 in the Ottawa River near Lima, Ohio. Levels of exposure were measured for petroleum by naphthalene-type metabolites, combustion by-products by benzo[*a*]pyrene-type metabolites, coplanar organic compounds by ethoxyresorufin-*O*-deethylase (EROD) activity, and urea by blood urea nitrogen (BUN) levels. The four biomarkers analyzed proved effective in determining differences between reference and polluted sampling sites, between geographically close (<0.5 km) sites, and between sampling years at sites common in both years. Calculated exposure criteria levels of the polycyclic aromatic hydrocarbon bile metabolites were found to be a conservative approximation of levels from a designated reference site and could thereby permit comparison of biomarker levels of fish from the Ottawa River to a regional reference level. Polycyclic aromatic hydrocarbon bile metabolite and EROD activity levels were more reflective of spatial patterns of contamination than BUN, although all biomarkers indicated differences over time. Biomarkers from white suckers seemed to be more responsive in detecting changes in contaminant levels than the same biomarkers from common carp. Lower levels in 1996 of all biomarkers at many sites suggested lower exposures than in 1993 and could be indicative of some improvement over the period.

Keywords—Polycyclic aromatic hydrocarbon bile metabolites Ethoxyresorufin-*O*-deethylase Exposure criteria values Blood urea nitrogen Biomarkers

INTRODUCTION

The ability to use biomarkers as diagnostic tools and to evaluate environmental exposure requires that the biomarkers be able to detect exposures above background levels. To do this, defensible reference values of exposure are needed and the biomarker measurements must be sensitive enough to detect differences from these references in discrete populations. This study evaluated the performance characteristics of four biomarkers in a river known to be historically polluted with polycyclic aromatic hydrocarbons (PAHs) using two types of references, an upstream location, selected by best professional judgement as representing a least impacted condition for an ecoregion [1,2], and regional exposure criteria derived from reference sites throughout Ohio, USA [3].

The Ottawa River in northwestern Ohio was selected for study because it has a history of severe pollutant impacts, and at its highest degree of impairment during the 1960s, was devoid of fish for more than 37 miles (59.5 km), including the Auglaize River downstream from the confluence with the Ottawa River [4]. Although conditions improved dramatically with better wastewater treatment and stricter enforcement after the 1972 Clean Water Act, the river is still severely impacted by multiple point source, and nonpoint landfill leaching, inputs in and around the city of Lima, Ohio. The cumulative impacts between river mile (RM) 40.1 and RM 36.0 begin downstream from the Lima combined sewer overflows s(RM 40.1–37.7) and an abandoned oil refinery landfill (RM 37.8–37.6) and increase with the addition of effluents from the Lima wastewater treatment plant (WWTP) (RM 37.7–37.2), an oil refinery (RM 37.1 to 36.9), and a chemical plant (RM 36.9 to 36.0) (Fig. 1). The high pollutant loadings of these clustered point source discharges are exacerbated when dilution capacity is diminished during low-flow periods [4].

Multiple biomarkers may help provide insight into sources where complex stressors such as mixed contaminants may be present [5]. Analyses of four biomarkers were used in this case study. Bile, liver, and blood samples from white suckers (*Catostomus commersoni*) and common carp (*Cyprinus carpio*) taken in 1993 and 1996 from the Ottawa River were analyzed for benzo[*a*]pyrene (B*a*P)- and naphthalene (NAPH)-type metabolites, PAH bile metabolite concentrations, ethoxyresorufin-*O*-deethylase (EROD) activity, and blood urea nitrogen (BUN) concentration.

Measurement of the metabolites of xenobiotics in fish bile is a useful diagnostic aid for certain types of aquatic contaminants such as PAHs because PAH metabolites accumulate in

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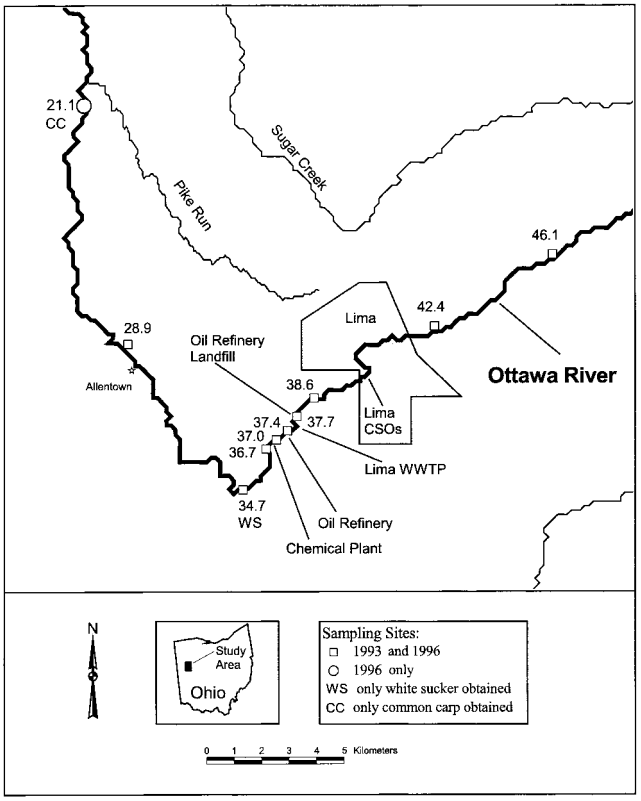

Fig. 1. Map of Ottawa River, Ohio, USA, showing sites where fish were sampled. Small inset shows location of study area (black square) in state of Ohio. Blow-up magnifies area in and below Lima, Ohio, with approximate location of point source inputs indicated. Star indicates site sampled in 1993. Open circle indicates site sampled in 1996. Star in circle indicates sites sampled in both years. Common carp (CC) and white sucker (WS) indicate sites where only these species were obtained; both species were obtained at all other sites.

bile in the gallbladder [6–8]. These biliary PAH metabolites can be measured using fixed-wavelength fluorescence [9] with exposures to PAHs estimated for petroleum sources with NAPH and from combustion sources with B*a*P. Polycyclic aromatic hydrocarbon measurements are important because PAHs with four or more condensed benzene rings have been identified as mutagenic and/or carcinogenic [10]. Elevated PAH levels in sediments [11,12] and bile [13] have been associated with the prevalence of neoplasia in fish.

Hepatic mixed-function oxygenase activity is induced in fish by exposure to organic contaminants such as PAHs and chlorinated hydrocarbons [14–16]. The mixed-function oxygenase activity is not induced by exposure to metals or radionuclides. The most common reaction used to measure hepatic mixed-function oxygenase activity catalyzed by cytochrome P4501A proteins involves the deethylation of the artificial substrate, 7-ethoxyresorufin, to form the fluorescent product resorufin. The EROD activity assay is a useful diagnostic tool because of its high sensitivity, simplicity, high specificity for P4501A-type enzymes, and low occupational hazard [17]. Numerous studies have indicated that EROD can be induced by anthropogenic contaminants such as PAHs, dioxins, and polychlorinated biphenyls [18–21]. Mixed- function oxygenase activity not only detoxifies but can also biotransform xenobiotics, notably PAHs, to reactive intermediates that can be highly toxic, mutagenic, or carcinogenic [22–24]. Ethoxyresorufin-*O*-deethylase assays have also proven sensitive enough to detect changes in contaminant levels over time [5,17,25,26] as well as space [27,28]. Techniques have been developed to quantify the EROD activity using fluorescence spectrophotometry [29] and ultraviolet–visible light spectrophotometry [30]. A much faster and simpler modification of this method, using a fluorescent plate reader, was used in this study and has been validated by Eggens and Galgani [31] against the well-established method of Burke and Mayer [29].

Blood urea nitrogen determination is used to measure the amount of nitrogenous waste, usually in the form of ammonia or urea, in fish blood. Elevated BUN levels in fish blood may be caused by high concentrations of nitrogenous compounds in water (nitrites, nitrates, ammonia), which occur in WWTP discharge zones, ammoniacal industrial chemical inputs, or fertilizer wash-in areas. Ammonia has also been indicated as a component of some toxic sediments [32]. High BUN levels may also result from damage to the gills (http://www.ornl.gov/ORNLReview/rev27-3/text/envmain.htm [accessed February 1998]) or kidneys [33,34] caused by acidic water or exposure to metals or phenols.

Each sublethal biomarker evaluates internal dose or early physiologic response to stressors that may be indicative of consequently higher-order health effects [25,26]. Estimation of external dose from biomarker response is impractical in field studies because of the presence, in addition to a suspected contaminant, of multiple environmental variables that may influence response. However, degree of exposure can be implied on a comparative basis in reference to a carefully selected reference population [3]. Measurements from field-sampled specimens may thus allow for assessment of the ability of the biomarkers, singly and in combination, to detect temporal and spatial patterns of contaminant distribution. Application of a suite of biomarkers has been advocated by many researchers because concurrent use increases the likelihood of contaminant detection and can reduce misinterpretation [21,27].

Analytical comparisons were made of levels of the four biomarkers from fish taken at downstream sites to those from an upstream reference site. Comparisons were made of biomarker levels between adjacent sampling sites. Levels of PAH bile metabolites from fish from all sites were compared to calculated exposure criteria values [3]. Comparisons were made between the same sites sampled by the Ohio Environmental Protection Agency (OEPA) in 1993 and again in 1996 to determine if conditions had changed in the Ottawa River. This study was undertaken to demonstrate the usefulness of biomarkers as indicators of exposure to common ecological stressors and to provide a practical demonstration of the analytical procedures for evaluating the level of exposure.

MATERIALS AND METHODS

Field collection

White suckers and common carp were collected by the OEPA at nine sites on the Ottawa River in September and October 1993 (Fig. 1). These two species were targeted as biomarker species because of their ubiquity [3], pollution tolerance, size for biomarker tissue harvesting, and benthivorous habits. The same two species were collected by the OEPA at many of the same sites in October and November 1996. Sampling sites were selected based on the longitudinal profile analysis method of the OEPA [1], which is generally designed to encompass sites upstream of a potential influence, at the point of initial mixing, in the recovery zone, and at a recovered site. In both sampling years, RM 46.1, located in a predominantly

agricultural area, was selected as a least impacted upstream reference site. Downstream sampling sites were focused between RM 38.6 and 34.7, the portion of the river in and around Lima where point source inputs are concentrated. Fish were collected using wading or boat method pulsed direct current electrofishing gear, and retained in a floating live well until tissue samples could be taken.

The number of carp and white suckers collected, bled, and necropsied was dependent on the availability of fish of the appropriate size. A minimum fish length of 120 mm, based primarily on tissue size requirements for assays, was targeted for collection, with no maximum size set. Age was not a consideration because the biomarkers used, unlike body burden, exhibit short-term change [17,35]. No sex ratio was targeted for collection. The number of fish from each site assayed for biomarkers in both years ranged from 5 to 10 white suckers and 2 to 10 common carp. Fish were anesthetized in MS222 (250 mg/L), and length and weight were recorded. Blood was drawn from the caudal vein through a 21-gauge needle into heparin-treated 3-ml blood drawing tubes. Whole blood was centrifuged on site at 3,000 rpm for 3 min; the plasma was removed, and then frozen at −100°C in a liquid nitrogen dry-shipper. The gall bladder was removed and nicked at the anterior end, and the bile was drained into an amber-colored microfuge tube. This tube was then frozen in a liquid nitrogen dry-shipper. Next, the liver was excised, wrapped in aluminum foil, and frozen in a liquid nitrogen dry-shipper. The frozen tissue samples were then transported to the National Exposure Research Laboratory of the U.S. Environmental Protection Agency, Cincinnati, Ohio. A chain of custody was maintained by logging samples into a standardized tracking system. The frozen samples were then transferred to −80°C freezers until analysis.

Bile metabolites

The NAPH-type and B*a*P-type metabolites present in the bile samples were measured using the methods of Lin et al. [9]. All standards and bile samples were handled under yellow lighting to prevent photodegradation of PAHs, bile, and biliary PAH metabolites. Diluted bile samples for analyses were covered with aluminum foil and stored at 4%C for up to 4 days or at −20°C for long-term storage. Polycyclic aromatic hydrocarbon bile metabolite levels were expressed as microgram equivalents per milligram protein based on a standard curve of the fluorescence of NAPH standards, analyzed at excitation/emission wavelengths of 290/335 nm, and B*a*P standards, analyzed at excitation/emission wavelengths of 380/430 nm.

Exposure criteria values

Regional exposure criteria values were determined for each type of metabolite for both white suckers and common carp following the methods described in Cormier et al. [3]. One-sided 95% upper reference values were estimated, following the recommendations of the International Federation of Clinical Chemistry [36], from all fish of each species sampled in 1992 to 1995 from OEPA reference sites. The exposure criteria values thus selected were, for white suckers, 0.5 μg/mg protein for B*a*P-type metabolites and 80 μg/mg protein for NAPH-type metabolites, and for common carp, 0.4 μg/mg protein for B*a*P-type metabolites and 130 μg/mg protein for NAPH-type metabolites.

*Ethoxyresorufin-*O*-deethylase*

Hepatic microsomes were prepared by differential centrifugation using the method of Lin et al. [37] with the following modifications. The homogenizing buffer contained 0.15 M KCl instead of 1.15%, and the storage buffer contained 25% glycerol versus 20%. The glycerol content was increased to adjust the stability of the buffer, originally used for rodents, to compensate for the lower stability of fish microsomes.

Ethoxyresorufin-*O*-deethylase activity was determined by a kinetic measurement of resorufin formation and results were expressed as picomoles (pmol) of resorufin produced per milligram of protein per minute. The incubation mixture for the EROD assay consisted of 0.1 M Tris buffer, pH 7.8, 5 mM magnesium chloride, 4.0 mM glucose-6-phosphate, 0.9 mM NADP, 0.5 IU/ml of glucose-6-phosphate dehydrogenase, microsomal protein, and 7.5 M of the artificial substrate 7-ethoxyresorufin. This mixture was added to a 96-well plate. Two replicates were used for each microsomal sample. Immediately after addition of the artificial substrate, the plate was analyzed on a Cytofluor 2350 fluorescent microtiter plate reader (Astroscan, Millipore, Isle of Mann, UK). No incubation period was included. The reaction product, resorufin, was determined spectrofluorometrically every 60 s for 15 min with excitation/emission wavelengths of 530/590 nm. The period of linearity was determined. All nonlinear data were deleted and the rate of resorufin produced per minute was calculated with a time-course analysis macro in Microsoft® Excel version 4.0 (Redmond, WA, USA).

Protein

Protein concentrations for bile were measured, after incubating for 16 h at room temperature, using Pierce's BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Samples were analyzed on a Dynatech MR5000 microtiter plate reader (Dynatech Laboratories, Chantilly, VA, USA) at a wavelength of 550 nm. Protein concentrations for microsomes were measured by a Sigma microprotein assay (Sigma Diagnostics, St. Louis, MO, USA) on a Hitachi 704 auto-analyzer (Hitachi, Tokyo, Japan).

Blood urea nitrogen

Blood urea nitrogen determinations were performed on a Hitachi 704 automatic analyzer using Boehringer Mannheim analytical kits and reagents (Boehringer Mannheim, Indianapolis, IN, USA). The method for determining BUN levels is based upon the method of Talke and Schubert [38], and is designed specifically for automatic analyzers that permit kinetic measurements. The assay measures both free ammonia and ammonia enzymatically released from urea. Blood urea nitrogen concentration is expressed as milligrams of BUN per deciliter.

Statistical analyses

Statistical analyses were performed using SAS® version 6.12 (SAS Institute, Cary, NC, USA). Two-way analyses of variance were performed on log-transformed data with significance of contrast tested using the Bonferroni multiple comparisons method to compare mean biomarker levels between sites. In cases where data homogeneity and normality could not be satisfied by transformation, the nonparametric Dunn procedure was performed to make the same comparisons under the Kruskal–Wallis test [39]. Because either an increase or decrease of level was of interest, a two-tailed analysis was

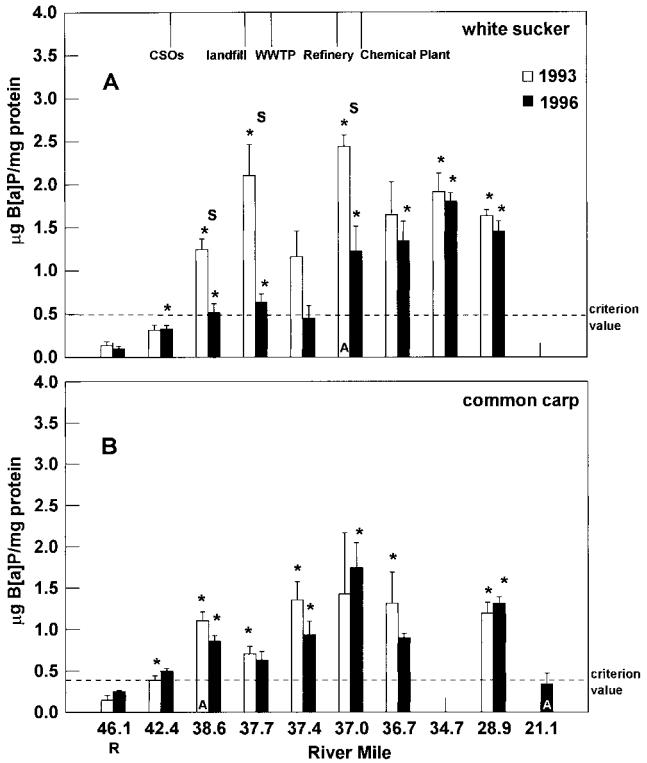

Fig. 2. Levels of benzo[*a*]pyrene (B*a*P)-type metabolites (μg B*a*P equivalents/mg protein) in bile of white suckers (**A**) and common carp (**B**) captured from the Ottawa River, Ohio, USA, in 1993 and 1996. Level of the estimated exposure criterion value is indicated by dashed horizontal line. Bars represent mean ± SE. * = significant difference between the upstream reference site R (river mile 46.1) and the downstream site. S = significant difference between sampling years at the same site. A in bar = significant difference from the nearest upstream site sampled in the same year ($p < \alpha$ star, where α star = 0.10/number of comparisons; Bonferroni multiple comparisons [parametric] or Dunn's procedure [nonparametric]). Approximate locations of point source inputs are noted at the top of each figure.

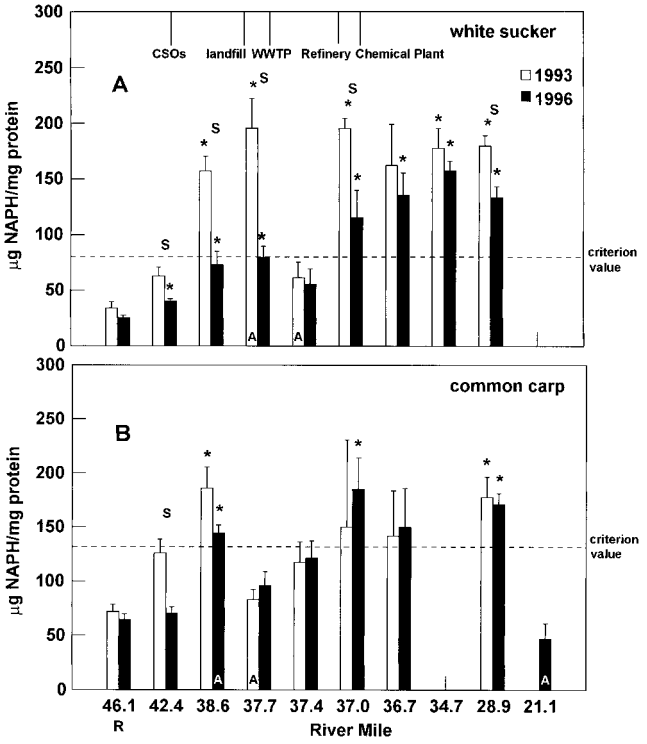

Fig. 3. Levels of naphthalene (NAPH)-type metabolites (μg NAPH equivalents/mg protein) in bile of white suckers (**A**) and common carp (**B**) captured from the Ottawa River, Ohio, USA. Level of the estimated exposure criterion value is indicated by dashed horizontal line. Other details are as noted in Figure 2.

performed controlling type I error rate (α) at 0.10. The α star was set at 0.10/*n* where *n* = the number of comparisons made. If *p* was found to be less than α star the difference between means was considered to be significant.

RESULTS

Bile metabolites

The B*a*P levels in white suckers were higher at all eight downstream sites than at the upstream reference site and significantly higher at many of the sites (most notably downstream of refinery and chemical plant outfalls), in both sampling years (Fig. 2A). Metabolite levels in fish at RM 37.0 were significantly higher than the adjacent upstream site in 1993. All white suckers at the upstream reference site had B*a*P-type metabolite levels lower than the estimated criterion value, whereas at least some white suckers from all the downstream sites had levels greater than or equal to the criterion value in both 1993 and 1996. The B*a*P-type metabolite levels in white suckers were lower in 1996 at most sites and significantly lower than in 1993 at RM 38.6, 37.7, and 37.0.

The B*a*P-type levels from common carp (Fig. 2B) were likewise higher than the upstream reference at all downstream sites, and significantly higher at several sites. The pattern of highest elevation for carp started below the WWTP outfall. All carp at the upstream reference also had B*a*P levels below the criterion value. All downstream sites, with the exception of RM 37.0, had at least some carp with levels above the value. No significant differences were found in common carp B*a*P metabolite levels at any site between the two sampling years. Differences between one set of adjacent downstream sites were found in each sampling year.

The NAPH-type metabolite concentrations from white suckers were elevated at all downstream sites relative to the upstream reference site in both sampling years and were significantly higher at five of eight sites in 1993 and seven of eight in 1996 (Fig. 3A). Significant differences in NAPH levels were detected between two sets of adjacent sites in 1993. All white suckers from the upstream reference site and also RM 42.4 had metabolite concentrations below the estimated criterion value, whereas at least some white suckers from all other downstream sites were above the estimated reference value in both sampling years. White sucker mean NAPH-type metabolite levels were significantly lower in 1996 than in 1993 at five downstream sites.

The NAPH-type metabolite levels of common carp were elevated downstream of the reference site with the exception of RM 21.1 in 1996 (Fig. 3B). Mean levels were significantly higher than those at the reference site at only two downstream sites in 1993 and three in 1996. Differences were detected between one set of adjacent sites in 1993 and two sets in 1996. All carp from the upstream reference site and also from RM 37.7 in 1993, and from RM 46.1, 42.4, and 21.1 in 1996, had NAPH levels below the estimated criterion value. At least some carp from all other sites exceeded the criterion value. As was the case with B*a*P, common carp showed little difference between years in NAPH metabolite level, being significantly lower in 1996 than 1993 at only one site, RM 42.4.

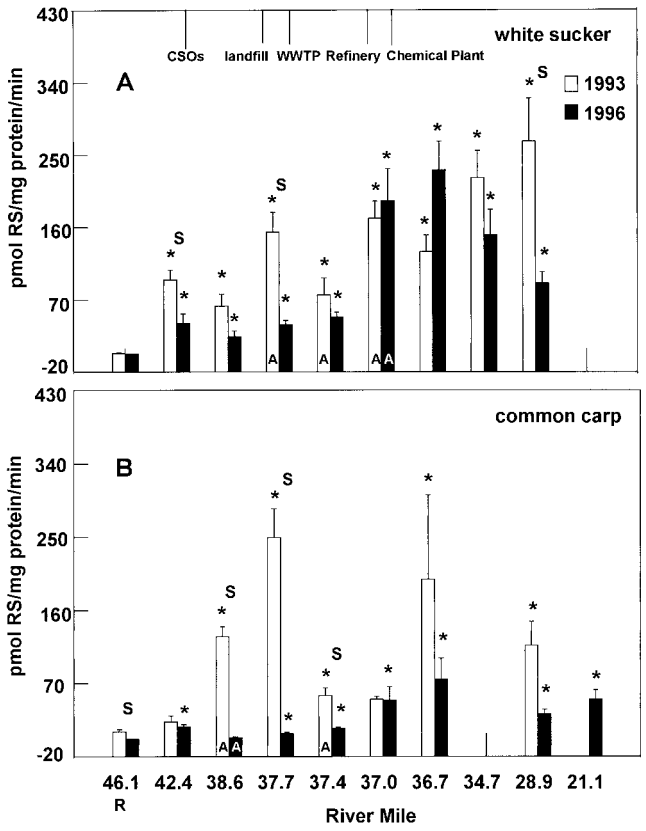

Fig. 4. Activity of ethoxyresorufin-*O*-deethylase (pmoles of resorufin/mg microsomal protein/min formed) in liver of white suckers (**A**) and common carp (**B**) captured from the Ottawa River, Ohio, USA. Other details are as noted in Figure 2.

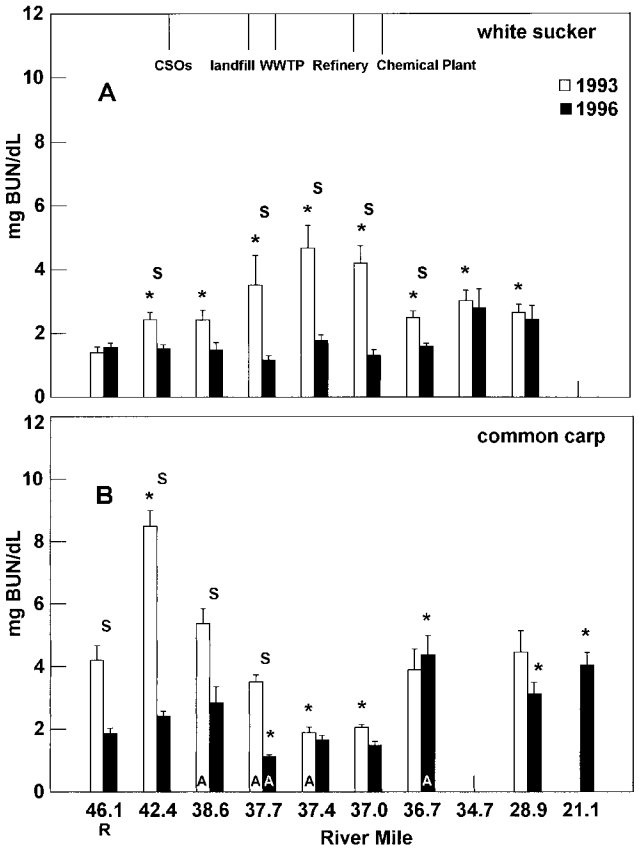

Fig. 5. Concentration of blood urea nitrogen (mg BUN/dl plasma) in blood of white suckers (**A**) and common carp (**B**) captured from the Ottawa River, Ohio, USA. Other details are as noted in Figure 2.

Ethoxyresorufin-O-*deethylase*

Ethoxyresorufin-*O*-deethylase activity levels for white suckers were significantly higher than at the upstream reference site at all downstream sites in both sampling years (Fig. 4A). Significant differences were found in white sucker EROD activity levels between three sets of adjacent sites in 1993 and one set in 1996. The EROD activity of white suckers was significantly lower in 1996 than in 1993 at RM 42.4, 37.7, and 28.9.

Common carp EROD levels (Fig. 4B) were significantly higher than at the upstream reference at five of seven downstream sites in 1993 and seven of eight sites in 1996. Differences were found between two sets of adjacent downstream sites in 1993 and one set in 1996. Common carp EROD activity was significantly lower in 1996 than in 1993 at the upstream reference site and at three contiguous sites, RM 38.6, 37.7, and 37.4.

Blood urea nitrogen

The BUN concentrations in white suckers were significantly higher than at the upstream reference site in fish from all downstream sites in 1993, whereas in 1996, no downstream sites had mean concentrations significantly elevated above the reference (Fig. 5A). No significant differences were detected in BUN levels of white suckers between adjacent sites in either sampling year. White sucker BUN levels were significantly lower in 1996 than in 1993 at five of eight downstream sites.

Common carp BUN levels in 1993 were significantly higher than at the upstream reference at RM 42.4 and significantly lower at RM 37.4 and 37.0 (Fig. 5B). The BUN levels for common carp in 1996 were significantly lower than the reference site level for fish from RM 37.7 and significantly higher than the reference level for fish from RM 36.7, 28.9, and 21.1. Significant differences were found in carp BUN levels between three sets of adjacent sites in 1993 and between two sets in 1996. Common carp BUN levels were significantly lower in 1996 than in 1993 at the upstream reference site and at three downstream sites.

DISCUSSION

Biomarkers for petroleum, combustion by-products, coplanar aromatic hydrocarbons, and ammonia were able to detect spatial and temporal differences in exposures of white suckers and common carp. Two key and distinct types of references helped to establish the degree of contamination. The upstream reference site permitted downstream sites to be compared within the river itself and regional exposure criteria values provided the context for exposure from a regional perspective.

In this case study, fish in the Ottawa River were shown to have high levels of exposures. In fact, mean levels for white sucker B*a*P-type (Fig. 2) and NAPH-type (Fig. 3) metabolites at sites RM 37.0, 34.7, and 28.9 exceeded the background level [3] by two to three times and for common carp B*a*P-type metabolites exceeded the background level by two to three times at RM 38.6, 37.4, 37.0, and 28.9. Mean levels for NAPH-type metabolites from common carp were in excess of background at RM 37.0 and 28.9.

In 1993 sampling, B*a*P-type metabolite levels in both spe-

cies increased sharply around RM 38.6, downstream of the Lima combined sewer overflows, peaked at RM 37.0, immediately downstream of the oil refining facility, and remained high to Allentown, Ohio, at RM 28.9 (Fig. 2). Some downstream dilution seemed to occur. The B*a*P levels in 1996 frequently mirrored 1993 spatial patterns and were consistently lower in white suckers. White sucker B*a*P levels peaked further downstream (RM 34.7) in 1996 than in 1993 but carp levels again peaked at RM 37.0.

White sucker NAPH-type metabolite levels peaked further upstream (RM 37.7) in 1993 than B*a*P levels (Fig. 3). White sucker NAPH levels at RM 37.7 were significantly higher than at RM 38.6 and could potentially have been influenced by landfill seepage in addition to combined sewer overflow inputs. Carp showed a peak in 1993 further upstream than white sucker, above the landfill but still within the combined sewer overflow outfall range, at RM 38.6 where levels were significantly elevated above the adjacent upstream site RM 42.4. White sucker NAPH levels in 1996 were highest at RM 34.7, as they had been for B*a*P. Carp levels peaked again further upstream than white suckers at RM 37.0. As was the case with B*a*P, 1996 NAPH-type metabolite levels somewhat mirrored those of 1993 and were again consistently lower in white suckers, with carp showing little difference between years. These spatial and response level discrepancies between species could be due to differences in foraging range and strategy or metabolism.

Similarities in spatial patterns of levels of both metabolites were noted for white suckers for both years, with an initial downstream rise in levels and then a decrease at RM 37.4 followed by a subsequent sharp increase at RM 37.0. The decrease could be attributed to the dilution effects of the Lima WWTP effluent just upstream at about RM 37.7 and the increase potentially attributed to effluent inputs, resultant from fuel, oil, coke, and chemical manufacture [4], from the oil refinery immediately upstream. In fact, 1993 levels of both metabolites in white suckers were significantly lower at RM 37.4 than at 37.7 and significantly higher at RM 37.0 than at 37.4. Carp levels of both metabolites showed a similar decrease, only slightly further upstream than white suckers, at RM 37.7 coincidental with the start of the WWTP outfall. This decrease was significant in 1993 carp NAPH levels. It should be noted that a series of eight low-head dams impound the Ottawa River from RM 43.5 to RM 38.0. Another dam is located downstream at Allentown, Ohio (RM 28.9). This may contribute to settlement of contaminants and may restrict fish migration as well, thus resulting in increased levels of detected contaminants in fish. However, the only point source outfalls located in the reach from RM 43.5 to 38.0 are the Lima combined sewer overflows.

Levels of PAH-type metabolites detected in fish bile have been linked to sediment levels of PAH contaminants [22,40]. Unpublished data, obtained through personal communication with the OEPA, indicated that sediment PAH concentrations, including those of B*a*P and NAPH, were elevated in 1996 between RM 34.5 and 38.6 of the Ottawa River (Table 1). These areas correspond to the areas adjacent to and downstream of point source discharges outside the town of Lima, Ohio, indicating that elevated levels of both B*a*P- and NAPH-type compounds were biologically available to white suckers and common carp within and downstream of the known point source inputs. However, the exact nature of the relationship between sediment and bile metabolite levels could not be elucidated simply from these data, because the sediment collection was not synoptic with the fish collection. Simple Pearson correlation between white sucker or common carp metabolite levels and the log of total organic carbon-normalized sediment concentrations (from sites within 0.1 RM of each other) were not statistically significant (white sucker: $n = 10$, $r = 0.194$, $p = 0.59$; common carp: $n = 9$, $r = 0.402$, $p = 0.28$).

Table 1. Dry weight sediment concentrations (mg/kg) of polycyclic aromatic hydrocarbons (PAHs) in sediments of the Ottawa River, Ohio, USA, during 1996 (from Ohio EPA unpublished report, EAS/1997-12-6)[a]

River mile	B*a*P concn.	NAPH concn.	Total PAH concn.
8.12	<0.7	NM	ND
15.98	0.9	NM	8.1
22.94	<0.6	NM	ND
25.75	0.9	<0.6	10.3
28.87[b]	1.0	<0.8	8.1
31.03	0.7	<0.5	7.2
32.60	1.5	<0.6	16.6
34.55[b]	2.0	0.8	47.8
36.30	3.4	<0.6	40.2
36.80[b]	5.2	<0.9	75.6
37.00[b]	2.6	<0.6	35.0
37.30[b]	32.6	3.6	662.4
37.70[b]	2.5	<1.6	27.6
38.00	5.4	NM	61.0
38.63[b]	4.2	NM	44.8
42.61[b]	<0.7	NM	2.2
44.26	<0.6	NM	ND
45.97[b]	<0.5	NM	ND

[a] B*a*P = benzo[*a*]pyrene; NAPH = naphthalene; NM = not measured; ND = not detectable.
[b] Sediment sampling sites closest to fish collection sites.

However, comparison of bile metabolite levels from white suckers sampled in 1996 with those taken in 1993 does suggest a reduction in bioavailable PAHs at several sites over the intervening period. Although PAH bile metabolite levels from white suckers at most downstream sites were still elevated relative to the reference site and criteria values, levels at many of the downstream sites were lower in 1996 than in 1993. Common carp metabolite levels were not indicative of either improvement or degradation over the period.

Significantly elevated hepatic EROD activity in white suckers from all downstream sites in both 1993 and 1996, is indicative of a metabolic response brought about by exposure to coplanar aromatic compounds (Fig. 4). Polychlorinated biphenyls were not indicated to be a significant pollutant in this system [4]. Common carp EROD levels, although not as definitive, showed a response pattern with significantly higher levels than the reference site at four sites in 1993 and seven sites in 1996. The EROD activity levels in white suckers showed some similarity between years in changes by RM. Activity patterns in 1993 were somewhat reflective of trends in bile metabolite levels, most notably with levels significantly lower at RM 37.4 than at 37.7 and significantly higher at RM 37.0 than at 37.4. However, sites of highest EROD activity levels did not correspond to sites of highest metabolite levels. White sucker EROD levels in 1996 were less well correlated to bile metabolite levels than 1993 levels. Such spatial similarities were not noted in common carp EROD levels either between the two sampling years or between EROD levels and levels of bile metabolites. However, responses of both species were similar in 1996, when activity levels increased at RM

37.0 (significantly in white suckers), below the oil refinery and peaked at RM 36.7, below the chemical plant. Lower activity levels far upstream and downstream of the multiple point source inputs indicate that the metabolic response noted could be spatially linked to these inputs as well as to effects of impoundment. The EROD activity in both species, although still elevated at many downstream sites compared to the reference site, was lower in 1996 than in 1993 at several downstream sites. These lower levels of induction could be, in part, reflective of the concomitant decrease in PAH bile metabolite levels.

Detected BUN levels did not show the clear spatial trends in response to point source inputs that were detected for bile metabolite levels and EROD activity (Fig. 5). However, some spatial associations may be inferred from the BUN data. The BUN assay is more sensitive to nitrogen loading, acidification, or excessive sediment metal levels than to complex anthropogenically generated organics. In this respect, BUN analysis provides information not given by the other assays. White sucker BUN levels were significantly elevated downstream in 1993 at several sites that were below point source inputs. The highest level in 1993 was at RM 37.4, just downstream of the Lima WWTP input (RM 37.7), an area expected to have elevated concentrations of nitrogenous compounds. Carp BUN levels for 1993 proved to be more cryptic than those of white suckers, with a high level at RM 42.2, upstream of most point source inputs and a level significantly lower than the upstream reference, as well as the adjacent upstream site, at RM 37.4, a site below the WWTP input. Agricultural nonpoint sources such as wash-in of fertilizer or livestock waste could have contributed to the inflation of upstream levels. Low-head dams upstream (RM 43.5 and 42.9) and just below (RM 42.3) the sampling site could have acted to concentrate such inputs. White sucker BUN levels in 1996 were not different than the reference site at any downstream site. The high BUN level in this year was further downstream (RM 34.7) than in 1993 and could be reflective of influences from any or several of the upstream inputs, although the most immediately upstream outfall is the chemical plant, which produces both nitrogenous and metal-containing effluents [4]. In 1996 BUN levels for carp also peaked at RM 36.7, downstream of the chemical plant, and were significantly elevated above the adjacent upstream site, RM 37.0.

Polycyclic aromatic hydrocarbon bile metabolite biomarkers proved efficient in many cases in differentiating between the upstream reference and downstream contaminated sites. Furthermore, when calculated regional exposure criteria values for B*a*P and NAPH were exceeded at a site, the site usually was also significantly elevated above the upstream reference site level. Metabolite levels also seemed to change in response to known point source influences. The EROD activity levels were often complementary to the bile metabolite values and were even more efficient at differentiating downstream contaminated sites from the upstream reference. The BUN concentrations were not as effective as the other biomarkers in discriminating the reference from the downstream sites, but this biomarker provides information on both point and nonpoint sources of contaminants, such as organic carbon and nitrogen enrichment. All biomarkers proved to be sensitive in a small geographical area with significant differences in mean levels detected between adjacent sites less than 0.5 km distant.

Biomarker measures from white suckers seemed more directly responsive to assumed point source influences and also showed a more complementary relationship between types of biomarkers than those from common carp. As mentioned above, this could be an effect of between-species differences in metabolism, migration (although the presence of low-head dams would, in some instances, confound this), or foraging strategies. Vigano et al. [21], in a study utilizing three cyprinids, also found species response differences, with barbel determined to be more responsive (via EROD induction) to contamination than nase or chub. These researchers attributed this difference to the exclusively benthic feeding of the barbel. These types of variables are often compensated for by in situ caging exposure experiments [25], an option not practically applicable in geographically extensive biomonitoring programs. For purposes of detection, fish age and residence time at a site would not seem to be critical issues in assessment of bile metabolites, because PAHs are rapidly metabolized and excreted [35], or EROD, which can change in response level in a relatively short period of time with change of exposure level [17]. Therefore, both biomarkers should reflect recent association with suspected contaminants. However, the BUN level can be indicative of both recent (change in concentration of nitrogenous compounds) or chronic (organ damage to gills or kidneys) exposure. Further, if movement was extensive, exposures would be uniform and significant differences would not have been detected between closely spaced sites. This was not the case. Overall results indicate that the preferred monitoring species of the two surveyed would be white sucker, because biomarkers of this species responded most logically to assumed influences and showed more between-year differences. Nonetheless, common carp assays could and did indicate significant elevation of bile metabolite and EROD activity levels at many downstream sites.

Even though levels of all biomarkers were still elevated in 1996 relative to the reference site and exposure criteria values, they were significantly lower at many sites compared to 1993. No sites had significantly higher levels in 1996, suggesting possible improvement in river conditions over the period. Additionally, levels of PAH metabolites and EROD activity changed similarly. Because no in-stream remediation efforts were noted during this period, changes can only be attributed to natural recovery and continued compliance and enforcement efforts. If correlated to like improvements in other measures, such as community metrics or sediment contaminant levels, the biomarker results may indicate some improvement in ambient contaminant levels at several sites in the Ottawa River over the period.

Despite the elements of uncertainty regarding between-species differences in movement or response level, the biomarkers revealed a distinct longitudinal gradient in response to suspected influences. Comparison of biomarker levels of fish from stream locations known to be contaminated to in-stream and regional references demonstrated that those fish were exposed well above background levels. Differences were also detected between the sampling periods. The biomarkers were thus shown to be effective in detecting exposure to contaminants. The information gained through such field studies can direct more intensive measurement of levels of detected contaminants or stressors or, by repeated measurement, monitor trends over time.

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