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Contaminated Sediments from Tributaries of the Great Lakes: Chemical Characterization and Carcinogenic Effects in Medaka (*Oryzias latipes*)

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Abstract. Sediments from four inshore industrial sites and a reference site in the Great Lakes were extracted with organic solvents to produce a crude extract, which was separated on alumina into two fractions: predominantly polycyclic aromatic hydrocarbons; and predominantly nitrogen-containing polycyclic aromatic compounds. Crude extracts were redissolved in acetone and analyzed by gas chromatography and gas chromatography-mass spectrometry. The acetone-redissolved crude extracts from the four industrialized sites contained 5.6–313.3 μg total polycyclic aromatic compounds/g sediment and 3.0–36.4 μg other compounds/g sediment. In addition to the typical EPA priority pollutants, a substantial amount (228.7 μg/g sediment) of alkyl-polycyclic-aromatic compounds was detected in sediments from one of the industrialized sites. Extracts from the reference site contained 1.55 μg total polycyclic aromatic compounds/g sediment. Medaka (*Oryzias latipes*) were exposed to multiple pulse doses of acetone-redissolved extracts and fractions. Medaka were also exposed to a known carcinogen, methylazoxymethanol acetate, to verify that chemicals produced tumors in the test fish. Acetone-redissolved extracts and fractions from contaminated sediments were toxic to medaka. Fin erosion and non-neoplastic liver abnormalities were more prevalent in medaka after exposure to acetone-redissolved extracts and fractions from contaminated sediments. Neoplasms previously associated with chemical exposure in wild fishes were induced in medaka exposed to acetone-redissolved extracts and fractions from two of the contaminated sites, but not from the reference site or controls. These findings further support the hypothesis that chemical contaminants in sediments are involved in epizootics of neoplasms in wild fishes at contaminated sites.

Fishes, particularly brown bullheads (*Ictalurus nebulosus*), from tributaries of the Great Lakes that are contaminated by polycyclic aromatic compounds (PACs) exhibit high incidences of neoplasms (Baumann *et al.* 1987; Baumann 1989; Black *et al.* 1980; Black 1983, 1984; Harshbarger and Clark 1990). Extracts of sediments from several of these contaminated tributaries were mutagenic in *in vitro* assays (Fabacher *et al.* 1988; Maccubbin *et al.* 1987; West *et al.* 1985, 1986).

Exposure of fishes to sediment-associated contaminants from Great Lakes tributaries in laboratory studies has also produced neoplasms and other abnormalities. Epidermal hyperplasia and multiple epidermal papillomas developed in some brown bullheads exposed by skin painting to PAC-containing sediment extracts from the Buffalo River, Erie County, New York (Black *et al.* 1985). In the same study, grossly visible nodules also developed in the livers of bullheads fed a diet containing Buffalo River sediment extract, and one fish had a large cholangioma. In rainbow trout (*Oncorhynchus mykiss*), hepatocellular neoplasms (basophilic focal alterations), eosinophilic focal alterations, and megalocytic hepatocytes were produced 12 months after microinjection of sediment extracts from the Black River, but not the Buffalo River, into eyed-stage eggs; however, the incidence of these lesions was low (Maccubbin *et al.* 1987). Sediment extract from Hamilton Harbour (an embayment in western Lake Ontario) containing high levels of polycyclic aromatic hydrocarbons (PAHs) was mutagenic in the Ames bacterial mutagenicity assay and induced hepatocellular carcinomas in rainbow trout exposed by microinjection of dimethylsulfoxide (DMSO)-solubilized sediment extracts into sac-fry (Metcalfe *et al.* 1988).

The present study was designed to further examine the role of chemicals in sediments in epizootics of neoplasms in Great Lakes fishes. Using a multiple pulse-dose protocol, medaka (*Oryzias latipes*) were exposed to the sediment extracts and fractions from five Great Lakes tributaries that were characterized chemically and tested for mutagenicity in a prior study (Fabacher *et al.* 1988). Medaka were selected because of their small (<4 cm) size (a longitudinal section of an entire fish can be placed on a microscope slide), history of use as a model for chemical carcinogenesis, and comparatively short (~6 months) latent period prior to tumor formation (Aoki and Matsudaira 1977; Harada *et al.* 1988; Hatanaka *et al.* 1982; Hawkins *et al.* 1985, 1986,

1988a, 1988b, 1988; Ishikawa *et al.* 1975; Metcalfe 1989). A similar exposure protocol was used to demonstrate the hepatic carcinogenicity of 7,12-dimethylbenz[*a*]anthracene (DMBA) in poecilids (Schultz and Schultz 1982). After the exposures, the fish were periodically sampled for carcinogenic effects, and the extracts and fractions were re-analyzed chemically to confirm previous findings and to document the nature of the material delivered to the test fish.

Materials and Methods

Sediment Collection, Extraction, Fractionation, and Analysis

Composite samples of sediments were collected in 1984 from four contaminated Great Lakes tributaries: the Black River at Lorain, Ohio; the Cuyahoga River at Cleveland, Ohio; the Menominee River at Marinette, Michigan; and the Fox River at Green Bay, Wisconsin. Sediments from Munuscong Lake, Michigan, which received only small amounts of chemical pollution, served as reference (Figure 1). Study sites were selected on the basis of where carcinogens were likely to be found in sediments as a result of industrial activities. The Black River, heavily contaminated by PACs including known carcinogens, was included as a positive field control. Composite sediment samples (about 35 L) were collected at each site with a Ponar dredge and frozen for later extraction and characterization. In the laboratory, the samples were air-dried, powdered in a blender, weighed, and extracted with organic solvents. The crude extracts were fractionated on neutral alumina to obtain A-2 (PAC) and A-3 (PACs containing nitrogen) fractions. Portions of the crude extract and fractions were packaged under nitrogen in sealed glass ampules for this study. Details of these procedures and additional information about the sites are given in Fabacher *et al.* 1988.

Stock solutions for fish exposures were prepared in acetone from the concentrated sediment extracts by opening the sealed glass ampules under gold lighting and rinsing the tar-like contents repeatedly with acetone. Residues in the ampules were further dissolved by sonication in a Bransonic®[1] 220 ultrasonic bath (Branson Cleaning Equipment Co., Shelton, CT) until no additional residues could be removed with acetone. Acetone supernatants were filtered through glass-fiber discs (Whatman Inc., Clifton, NJ, GF-D, 3-μm pore size) into foil-covered volumetric flasks. Acetone-insoluble residues were rinsed with methylene chloride into clean beakers and evaporated to dryness at room temperature. Additional acetone was added to the residues, sonication and filtration were repeated, and the filtered supernatants were added to the previous stocks. The remaining insoluble residues were weighed, the weights of the redissolved solutes were determined by differences, and concentrations of the stock solutions were calculated. Stock volumes were adjusted with acetone and solutions were refrigerated in black Teflon® bottles until use. The extracts and fractions were redissolved in acetone and the resulting stock solution concentrations were determined (Table 1).

Aliquots of stock solutions of acetone-redissolved crude extracts (ARCEs) were analyzed for PACs. During subsequent procedures, the ARCEs were stored in aluminum foil-wrapped containers in the dark and handled under low-light conditions to minimize photooxidation. All extracts, except those from Cuyahoga River sediments, contained small amounts of precipitate. The ARCEs were enriched by two consecutive gel permeation chromatography (GPC) procedures by two different instruments. The first consisted of an Autoprep® 1001 (ABC Laboratories, Columbia, MO) with a 40-cm × 2.5-cm (id) column packed with 50 g of Bio-Beads® S-X3 resin, 200–400 mesh (Bio-Rad Laboratories, Richmond, CA). The mobile phase was 1:1 (v:v) cyclohexane:dichloromethane pumped at 5 mL/min. Each of the sediment extracts was loaded into a 5-mL sample loop. The second GPC apparatus was a modification of the first in which a Waters Model 6000 A pump (Waters Chromatography, Milford, MA) had been substituted for the standard pump, and the capacities of the sample loops had been reduced from 5.0 mL to 1.0 mL. Two tandem columns (22-cm × 1-cm id) were each packed with 3.8 g of S-X3 (270–325 mesh). The mobile phase was 1:1 (v:v) cyclopentane:dichloromethane pumped at 1.3 mL/min. Through mixed separation mechanisms of size exclusion and adsorption, the GPC systems were used to remove interfering compounds such as aliphatic hydrocarbons, phthalate esters, and biogenic compounds, but selectively retain many classes of compounds of interest including PACs, polychlorinated biphenyls (PCBs), organochlorine pesticides, and phenols (Ribick *et al.* 1981).

A Finnigan 4023 quadrupole GC/MS system equipped with a 30-m × 0.25-mm (id) DB-5 (0.25 μm film thickness) capillary column was used to perform the separations for detailed qualitative and quantitative analyses. Initial mass calibration was performed according to the instrument company's specifications. About 1 μL of the sediment extracts (final volume 250 μL) was direct-injected for GC/MS analyses. The GC oven was temperature-programmed for an initial temperature of 45°C, held 1 min, increased to 210°C at 7°C/min, increased again to 295°C at 5°C/min, then increased at 0.5°C/min to a final temperature of 300°C. The GC/MS system acquired full-scan, m/z 35–550 electron ionization mass spectra at the rate of 1 scan/sec.

Data from GC/MS analyses were processed with the INCOS™ system. Mass spectra of the several hundred detected GC peaks were retrieved, the background was subtracted, and, when possible, the peaks were matched with the 42,000-compound National Institute of Standards and Technology (NIST) spectral reference library (EPA/NIH Mass Spectral Data Base 1986). For peaks that did not match, mass spectral interpretation techniques were used. When possible, retention indices (Lee *et al.* 1981) were used to verify tentative identifications.

Quality Assurance of Extract Analyses

Each 2-g equivalent of extract and a procedural blank for this study were spiked with 0.5 μg/g of D_4-1,2-dichlorobenzene and each of seven perdeuterated PAHs (naphthalene, acenaphthene, fluorene, anthracene, pyrene, benz[*a*]anthracene, and chrysene) immediately before enrichment with the first GPC and designated potential procedural internal standards. To calibrate the elution profile of each GPC system and to assure chromatographic performance, bis(2-ethylhexyl)phthalate, 2,3,5,6,-tetrachloroterephthalic acid-dimethyl ester (Dacthal) and pyrene were analyzed with an inline UV detector (254 nm). A test mixture (Grob *et al.* 1978) containing 5 ng/μL of each compound was analyzed to evaluate both GC separation and MS sensitivity. A standard mixture of 51 PACs and organochlorine pesticides and 10 perdeuterated compounds at 1.9–8.5 ng/μL was analyzed to facilitate qualitative GC/MS confirmations and to establish quantitative response factors. For other compounds, probable identifications were made by MS NIST Library match. Tentative identifications were made by mass spectral interpretation, and unknown compounds were characterized by scan time and molecular weight (Christman 1984). Concentrations of other compounds were estimated by ion response factors relative to internal standards (D_8-naphthalene and D_{10}-pyrene). Detection limits were 0.01–0.02 μg/g for individual PACs and 0.02–0.39 μg/g for individual organochlorine pesticides and other compounds. The detection limit for total PCBs was 0.2 μg/g. The usual quantitation ion(s) responses for detection limits were ≥5 × signal/noise.

Identification of PACs with identical molecular weights was diffi-

[1] Use of trade names does not constitute government endorsement.

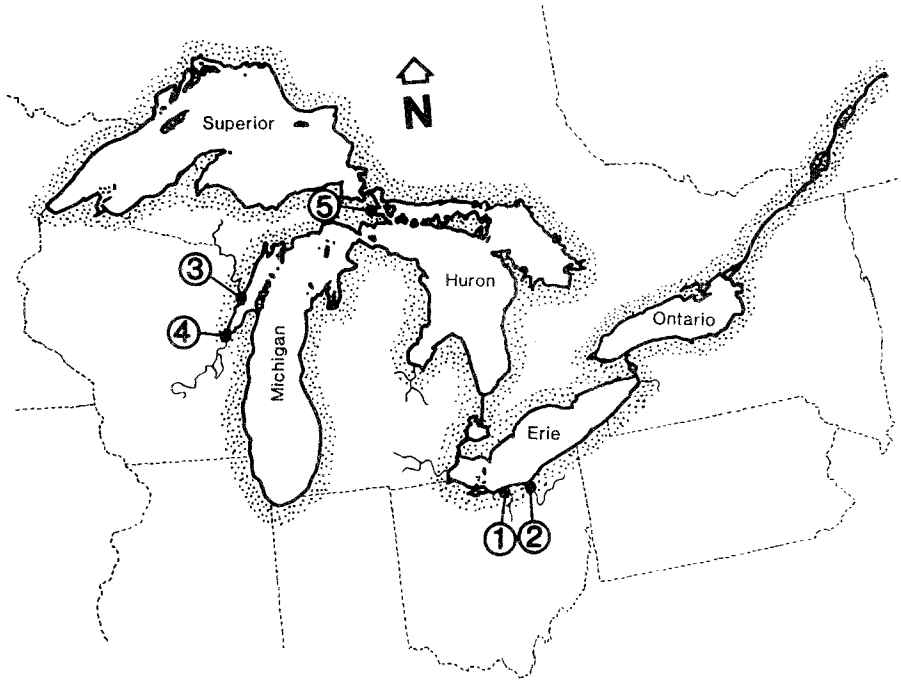

Fig. 1. The Laurentian Great Lakes of North America indicating sites from which sediments were collected.
1. Black R.; 2. Cuyahoga R.;
3. Menominee R.; 4. Fox R.;
5. Munuscong L.

Table 1. Solubility of sediment extracts and fractions and concentration of acetone stock solutions

Component and site	Extract solubilized (%)	Concentration of stock solution (mg/ml)
Crude extract[a]		
Black R.	91	5.7
Cuyahoga R.	87	7.0
Fox R.	91	10.3
Menominee R.	94	5.0
Munuscong L.	85	3.5
A-2 fraction		
Black R.	97	9.0
Cuyahoga R.	89	4.0
Fox R.	98	6.0
Menominee R.	86	4.0
Munuscong L.	87	1.0
A-3 fraction		
Black R.	88	4.8
Cuyahoga R.	89	4.0
Fox R.	90	5.4
Menominee R.	81	4.0
Munuscong L.	—[b]	—

[a] The amounts (g) of air-dried sediment extracted for Fabacher *et al.* (1988) were: Black River (BR), 1,350; Cuyahoga River (CR), 1,069; Fox River (FR), 687; Menominee River (MR), 1,628; Munuscong Lake (ML), 695. Resulting crude extracts (g) were: BR, 13.5; CR, 9.1; FR, 13.8; MR, 8.2; ML, 0.7. Aliquots (g) used to prepare crude extract stock solutions were: BR, 1.4; CR, 1.4; FR, 1.4; MR, 0.9; ML, 0.1

[b] Not enough produced

cult whenever mass spectra had similar fragmentation patterns. Some of these PACs were resolved by GC and identified by retention time whereas others, which either could not be separated or for which standards were not available, were summed (Table 2). In a few instances, our identified compound names differed from names reported by Fabacher *et al.* (1988). For example, in contrast to the tentative identification (Fabacher *et al.* 1988) of MW 218 compounds as methyl-phenyl naphthalenes, we differentiated methyl-phenyl naphthalenes from benzonaphthofurans with NIST library spectra (Footnote m, Table 2). Similarly, Fabacher *et al.* (1988) tentatively reported MW 266 compounds as dibenzofluorenes; we called them either methyl-benzofluoranthenes/pyrenes (Footnote s) or dibenzofluorenes, depending on whether there was an abundant $(M-1)^+$ ion found (West *et al.* 1986).

Only a few compounds were detected in the procedural blank. Biphenyl was present at 0.03 μg/g, whereas concentrations of naphthalene, fluorene, and phenanthrene were <0.03 μg/g. Compounds detected at >0.03 μg/g in the blank included tetrahydropyranone, dichlorocyclohexane, and saturated hydrocarbons. The source of the tetrahydropyranone is unknown. We have determined that dichlorocyclohexane and saturated hydrocarbons are typical impurities in the solvents used.

Quantitation was performed with a technique that self-corrected concentrations by recoveries of the internal standards. Although absolute recoveries of the internal standards were not determined, recoveries of most spiked deuterated PAHs, relative to D_{10}-pyrene, were 61–115%. Despite efforts to minimize photolytic degradation, relative recoveries of the two photolytically-sensitive PAHs (D_{10}-anthracene and D_{12}-benz[*a*]anthracene) ranged from <5% in the least contaminated ARCE to 110% in the most contaminated. Accompanying the loss of the two PAHs was the tentative identification of two photodegradation products: D_8-anthracenedione in the procedural blank and in the two least contaminated ARCEs; and D_{10}-benz[*a*]anthracenedione in the least contaminated ARCE (Table 4).

Fish Exposures

Methylazoxymethanol acetate (MAMA), a known carcinogen, was obtained from the Chemical Carcinogen Reference Standard Repository, Division of Cancer Etiology, NCI/NIH, Bethesda, MD 20205 (IIT Research Institute, Chicago, IL) for use as a positive control to verify that the test fish responded to chemicals with tumor formation. A stock solution in water (3.26 mg/mL) was prepared and refrigerated in a black Teflon® bottle.

Table 2. Concentrations (μg/g sediment on a dry weight basis) of PACs in acetone-redissolved crude extracts (ARCEs) from Great Lakes sediments compared to concentrations found in a prior study. Number of isomers or components summed are listed in parentheses

Compound name (# of Isomers)	MW[a]	ID[b]	Black R.		Cuyahoga R.		Fox R.		Menominee R.		Munuscong L.	
			Prior study[c] GC/FID	This study GC/MS	Prior study GC/FID	This study GC/MS	Prior study GC/FID	This study GC/MS	Prior study GC/FID	This study GC/MS	Prior study GC/FID	This study GC/MS
Polycyclic aromatic hydrocarbons												
{Naphthalene}[d]	128	C[e]	14.[f]	3.8	—[g]	0.08	1.5	2.2	—	0.36	0.003	0.12
2-Methyl Naphthalene	142	C	6.6	3.8	0.15	0.23	4.1	9.4	—	0.07	0.009	0.06
1-Methyl Naphthalene	142	C	3.4	2.3	0.10	0.13	3.5	7.0	—	0.06	0.005	0.04
C_2-Naphthalenes	156	C	4.3	3.5 (7)	—	1.3 (7)	18.	24.2[h] (7)	—	0.03 (2)	0.024	0.05 (2)
C_3-Naphthalenes	170	P[i]	—	5.8 (8)	2.0	4.3 (10)	12.	41.2 (10)	—	—	—	<0.02[j] (1)
C_4-Naphthalenes	184	P	—	3.2 (12)	—	3.2 (12)	—	23. (13)	—	—	—	—
C_5-Naphthalenes	198	P	—	—	—	—	—	1.0 (2)	—	—	—	—
Biphenyl	154	P	2.4	0.66	—	0.04	—	1.3	—	—	0.01	0.06
Methyl Biphenyls	168	P	—	0.47 (2)	—	—	—	1.4 (2)	—	—	—	—
C_2-Biphenyls	182	P	—	0.59 (3)	—	0.16 (3)	—	1.5 (3)	—	—	—	—
C_3-Biphenyls	196	P	—	0.7 (2)	—	—	—	4.5 (5)	—	—	—	—
C_4-Biphenyls	210	P	—	1.2 (4)	—	0.1 (1)	—	3.1 (3)	—	—	—	—
{Acenaphthylene}	152	C	17.	1.54	—	0.16	—	<0.06	—	0.14	—	<0.01
{Acenaphthene}	154	C	2.5	1.1	—	0.62	—	0.67	—	0.21	—	—
Methyl Acenaphthenes, Diphenylmethane or equivalent	168	T[k]	—	0.38 (3)	—	—	—	—	—	—	—	—
{Fluorene}	166	C	16.	3.0	—	0.67	2.6	1.5	0.17	0.04	—	0.015
Methyl Fluorenes	180	P	—	1.4 (3)	—	0.70 (2)	3.5	3.3 (2)	—	0.04 (1)	—	—
C_2-Fluorenes	194	P	—	1.0 (3)	—	0.93 (4)	—	0.90 (2)	—	—	—	—
{Phenanthrene}	178	C	52.	10.	4.7	4.6	4.5	8.7	1.9	0.06	0.77	0.095
{Anthracene}	178	C	15.	4.7	1.0	0.33	—	0.86	0.45	0.06[l]	—	—[l]
Methyl Phenanthrene/ Anthracenes	192	C	8.6	7.5 (5)	4.7	4.4 (4)	6.1	12.5 (4)	2.3	0.04 (2)	—	0.08 (4)
C_2-Phenanthrene/ Anthracenes	206	P	—	6.0 (11)	—	6.0 (11)	—	11.4 (12)	—	—	—	—
C_3-Phenanthrene/ Anthracenes	220	P	—	3.0 (10)	—	3.6 (12)	—	5.1 (9)	—	—	—	—
C_4-Phenanthrene/ Anthracenes	234	T	—	0.29 (2)	—	0.37 (1)	—	1.1 (2)	—	—	—	—
Cyclopenta[*def*]-phenanthrene	190	P	6.6	2.6	—	—	—	—	0.24	—	—	—

Phenyl Naphthalene	204	P	2.0	1.3	0.67	0.60	—	0.82	—	—	—	—	
Methyl-Phenyl Naphthalene[m]	218	P	5.8	0.26	2.2	0.15	—	0.41	0.98	—	—	—	
{Fluoranthene}	202	C	33.	13.	7.2	7.2	1.9	2.1	2.2	0.06	0.10	0.16	
Acephenanthrylene	202	P	6.0	1.6	—	—	—	0.06	0.29	<0.04	—	—	
{Pyrene}	202	C	24.	9.2	5.6	5.4	1.4	2.7	3.0	0.05	0.11	0.10	
Methyl-Fluoranthene/ Pyrenes and Benzofluorenes	216	P	14.4	7.6 (6)	2.4	4.0 (6)	2.9	6.4 (6)	7.6	<0.03 (1)	0.067	—	
C_2-Fluoranthene/ Pyrenes	230	P	—	2.0 (6)	—	0.80 (3)	—	5.5 (7)	—	—	—	—	
C_3-Fluoranthene/ Pyrene	244	T	—	0.14	—	—	—	0.22	—	—	—	—	
Cyclopenta[*c,d*]pyrene	226	P	2.9	1.1	—	0.83	—	0.30	—	—	—	—	
Benzo[*ghi*]fluoranthene	226	—	1.5	—	0.49	—	—	—	—	—	—	—	
11*H*-Benz[*bc*]aceanthrylene,[n] 4*H*-Cyclopenta[*def*]-chrysene or equivalent	240	T	2.9	1.1 (3)	—	—	—	—	—	—	—	—	
Benzo[ ]phenanthrene[o]	228	—	1.2	—	—	—	—	—	0.27	—	—	—	
{Benz[*a*]anthracene}	228	C	11.	6.6	2.2	2.3	0.70	3.2	0.94	0.07[p]	0.046	—[p]	
{Chrysene/Triphenylene}	228	C	10.	5.7	3.1	4.1	1.0	2.6	1.3	0.065	0.069	0.14	
Methyl-Chrysene/Triphenylene/ Benz[*a*]anthracene/ Benzophenanthrenes, other Methyl-PAH of $C_{19}H_{14}$ or Phenyl Fluorene	242	T	4.2	2.5 (7)	1.3	1.5 (4)	2.6	6.9 (6)	1.2	<0.03 (1)	—	—	
C_2-Chrysene/Benz[*a*] anthracenes	256	T	—	0.48 (2)	—	—	—	3.3 (5)	—	—	—	—	
{Benzofluoranthenes} and other Benzopyrenes[q]	252	P	14.9	16. (3)	4.6	10.1 (2)	1.9	9.1 (3)	1.2	0.15 (2)	0.091	0.27 (2)	
Benzo[*e*]pyrene	252	P	6.0	4.9	1.9	3.2	1.7	8.2	0.59	—	0.068	0.12	
{Benzo[*a*]pyrene}	252	C	8.8	4.4	2.6	0.16[r]	1.0	7.2	0.65	—[r]	0.044	—[r]	
{Perylene}	252	C	3.6	2.4	0.57	0.27	—	0.88	0.18	—	0.092	—	
Dibenzofluorenes[s]	266	T	1.6	1.3 (2)	—	0.3 (1)	—	3.8 (2)	0.37	0.30 (1)	—	—	
Methyl-Benzofluoranthenes, Methyl-Benzopyrenes	266	T	—	1.0 (3)	—	0.8 (3)	—	7.7 (5)	—	—	—	—	
{Indeno[1,2,3-*cd*]pyrene}	276	C	6.4	16.	1.4	9.0	—	3.7	0.18	1.9	—	<0.13	
Benzo[ ]chrysene, Dibenz[ ]anthracene, or Dibenzo[ ]phenanthrene	278	T	—	2.0	—	—	—	2.0	—	—	—	—	
{Dibenz[*a,h*]anthracene}	278	C	1.6	2.8	—	1.6	—	4.9	—	0.55	—	—	
Other Dibenzanthracenes or Dibenzophenanthrenes	278	P	—	2.8 (2)	—	—	—	3.0 (2)	—	<0.5 (1)	—	—	
{Benzo[*g,h,i*]perylene}	276	C	5.4	7.4	1.1	4.2	—	3.2	0.20	<0.07	—	—	
PAHs (MW 302)	302	—	9.5	—[t]	—	—[t]	—	—[t]	—	—[t]	—	—[t]	
Total PAHs			325.1 (Unknown)	182.1 (133)	50.0 (Unknown)	88.4 (107)	70.9 (Unknown)	254.1 (135)	26.2 (Unknown)	4.9 (26)	1.5 (Unknown)	1.5 (21)	
Nitrogen-containing PACs													
Quinoline	129	C	—	0.3	—	<0.03	—	—	—	—	—	—	

Table 2. (cont'd)

Compound name (# of Isomers)	MW[a]	ID[b]	Black R. Prior study[c] GC/FID	Black R. This study GC/MS	Cuyahoga R. Prior study GC/FID	Cuyahoga R. This study GC/MS	Fox R. Prior study GC/FID	Fox R. This study GC/MS	Menominee R. Prior study GC/FID	Menominee R. This study GC/MS	Munuscong L. Prior study GC/FID	Munuscong L. This study GC/MS
Benzo[ ]quinoline	179	P	0.48	—	—	—	—	—	—	0.05	—	—
Acridine	179	C	—	<0.1	—	—	—	—	—	0.05	—	—
Carbazole	167	P	3.9	0.74	—	0.29	—	0.40	—	0.13	—	—
Methyl Carbazoles or equivalent	181	T	—	0.13 (1)	—	0.05 (1)	—	0.74 (3)	—	0.06 (1)	—	—
C_2-Carbazole or equivalent	195	T	—	—	—	—	—	0.50	—	<0.04	—	—
Benzacridines or equivalent	229	T	0.67 (1)	0.42 (1)	—	0.34 (1)	—	—	—	0.24 (2)	—	—
Benzo[ ]carbazole or Phenanthreneamine/ Fluorantheneamine	217	U[u]	4.6 (3)	0.28 (1)	—	0.13 (1)	—	0.69 (1)	—	<0.04 (1)	—	—
Total N-PACs			9.7 (6)	2.0 (5)	—	0.7 (4)	—	1.6 (5)	—	0.6 (7)	—	—
Sulfur-containing PACs												
Methyl Benzothiophenes	148	P	—	—	—	—	—	2.0 (3)	—	—	—	—
C_2-Benzothiophenes	162	P	—	—	—	—	—	1.9 (3)	—	—	—	—
C_3-Benzothiophenes	176	P	—	—	—	—	—	5.2 (7)	—	—	—	—
C_4-Benzothiophenes	190	P	—	—	—	—	—	3.1 (5)	—	—	—	—
Dibenzothiophene	184	C	3.7	1.0	—	0.41	3.5	1.3	0.11	0.04	0.05	<0.01
Methyl Dibenzothiophenes	198	P	1.5	1.5 (3)	—	1.3 (3)	—	3.8 (4)	—	0.04 (2)	—	—
C_2-Dibenzothiophenes	212	P	—	2.6 (8)	—	2.5 (8)	—	2.9 (4)	—	<0.03 (1)	—	—
C_3-Dibenzothiophenes	226	P	—	3.4 (8)	—	2.4 (7)	—	<0.2 (1)	—	—	—	—
C_4-Dibenzothiophenes	240	P	—	0.49 (5)	—	0.16 (2)	—	<0.09 (1)	—	—	—	—
Benzo[b]naphtho[2,1-*d*]-thiophene[v]	234	C	1.2	1.1	—	0.78	1.0	1.5	0.31	—	—	<0.025
Other Benzo[*b*]naphthothiophenes	234	P	—	0.62 (2)	—	—	—	1.1 (2)	—	—	—	—
Methyl-Benzonaphtho-thiophenes or equivalent	248	P	—	0.66 (3)	—	0.51 (3)	—	5.5 (4)	—	—	—	—
C_2-Benzonaphthothiophenes or equivalent	262	P	—	—	—	0.08 (1)	—	3.0 (3)	—	—	—	—
Total S-PACs			6.4 (Unknown)	10.7 (31)	—	8.0 (24)	4.5 (2)	31.6 (39)	0.42 (2)	0.1 (4)	0.05 (1)	0.04 (2)

Oxygen-containing PACs												
Dibenzofuran	168	C	9.5	3.1	—	0.55	1.7	4.2	—	0.03	—	<0.015
Methyl Dibenzofurans	182	P	—	0.49 (3)	—	0.60 (2)	4.7	11.6 (3)	—	—	—	—
C_2-Dibenzofurans	196	T	—	—	—	0.3 (2)	—	3.7 (2)	—	—	—	—
Benzonaphthofurans	218	P	—	4.3 (5)	—	1.4 (3)	—	2.9 (4)	—	—	—	—
Methyl-Benzonaphthofurans	232	T	—	0.8 (2)	—	0.4 (2)	—	1.5 (2)	—	—	—	—
Total O-PACs			9.5 (1)	8.7 (10)	—	2.7 (8)	6.4 (Unknown)	23.9 (12)	—	0.03 (1)	—	—
Grand totals:												
EPA Priority pollutants			229.1 (16)	100.3 (16)	33.5 (11)	50.5 (16)	16.5 (9)	52.7 (16)	12.2 (12)	3.7 (14)	1.2 (9)	1.0 (9)
Other unsubstituted PACs			68.3 (18)	33.6 (27)	3.6 (4)	8.4 (15)	7.9 (3)	31.8 (21)	2.0 (7)	1.4 (11)	0.3 (5)	0.3 (6)
Alkyl-substituted PACs			53.8 (Unknown)	67.2 (136)	12.9 (Unknown)	41.6 (115)	57.4 (Unknown)	228.7 (154)	12.5 (Unknown)	0.57 (16)	0.1 (Unknown)	0.25 (9)
Grand Total of all listed PACs			351.2 (Unknown)	201.1 (179)	50.0 (Unknown)	100.5 (146)	81.8 (Unknown)	313.3 (191)	26.7 (Unknown)	5.6 (41)	1.6 (Unknown)	1.55 (24)

[a] Molecular weight
[b] Identification
[c] Fabacher et al. (1988). The concentrations from this prior study were from analysis of crude extracts and fractions rather than from ARCEs
[d] EPA 16 Priority Pollutant PAHs are indicated by brackets
[e] Confirmed identification by MS and GC retention times or indices using an analytical standard
[f] Individual compounds were quantitated to two significant places
[g] Not reported in prior study; not detected in this study
[h] Sum of compounds quantitated to two significant places
[i] Probable identification by MS NIST Library match
[j] Detected below the limit of quantitation
[k] Tentative identification by MS interpretation
[l] Probably degraded by photooxidation, since most of D_{10}-anthracene internal standard was also degraded
[m] Prior study reported unknown MW 218 PAHs may be methyl-phenyl-naphthalene. For other MW 218 compounds, see also benzonaphthofurans.
[n] Prior study reported unknown MW 240 PAHs may be cyclopentabenz[*a*]anthracene; our identifications are consistent with West et al. (1986), p. 243 in that each mass spectrum had an abundant $(M-1)^+$ ion.
[o] Unspecified isomers
[p] Probably degraded by photooxidation, since most of D_{12}-benz[*a*]anthracene internal standard was also degraded
[q] Sum of Benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and other benzofluoranthenes plus other unspecified benzopyrenes
[r] Probably degraded by photooxidation
[s] Dibenzofluorenes were differentiated from other MW 266 PAHs by whether each mass spectrum showed an abundant $(M-1)^+$ ion (West et al., 1986, p. 243)
[t] For this study, GC/MS acquisition conditions (GC temperature program and analysis time) may have prevented their detection
[u] Identification is unknown
[v] Prior study inadvertently reported isomer [1,2-*d*]

Juvenile medaka were cultured at our laboratory according to methods provided with the brood stock (Carolina Biological Supply, Burlington, NC). Adult medaka were fed flake food (Tetra Min®, Tetra Werke, West Germany) and live brine shrimp (*Artemia salina*); newly-hatched medaka were fed an infusoria (protozoan) culture until they were large enough to eat brine shrimp. Vigorous 5–9 d-old juveniles (<0.5 cm total length) with brine shrimp visible in their guts were separated 24 h before exposures.

Pulse-dose exposures were conducted in a vented glove box. Each exposure consisted of 5–7 dosage groups: 3–5 doses of ARCEs or acetone-redissolved A_2 (ARA-2F) and A_3 (ARA-3F) fractions; controls and carrier (acetone) controls; and positive (MAMA) controls. The concentrations of ARCEs, ARA-2Fs, and ARA-3Fs in the exposures (Table 5), chosen to avoid excessive mortality, were determined after conducting range-finding tests for acute toxicity. Because of the small quantity of Munuscong Lake extract produced, no ARA-3F was made, and only low doses could be made for the ARCE and ARA-2F exposures. Four 24-h pulse doses were conducted at weekly intervals for three replicate groups of 50 fish per dosage group. Each group was held in a separate exposure chamber (1000-mL beaker with stainless steel screen bottom) throughout the series of pulse doses. Exposure solutions were prepared by adding stock solutions to 1000 mL of water (equilibrated to 26°C) in 2,000-mL beakers. One chamber from each dose group was transferred in well water from 50-L aquaria and suspended in exposure solution for 24 h. After this period, exposure chambers were removed from the exposure solution, rinsed gently, and returned to the 50-L aquaria. The fish were fed brine shrimp daily except during the exposures when they were not fed. This procedure was repeated for the other two sets of replicates at 2-d intervals, and the cycle of pulse doses was repeated weekly until each replicate had received four doses. Positive controls received a single 24-h exposure to 2 mg/L MAMA during the fourth pulse dose period.

After exposure, survivors were transferred to clean exposure chambers and suspended in the holding aquaria at 26°C (one aquarium per dose group). After 30 d, fish were released from the exposure chambers into compartments in the aquaria separated by perforated plastic sheeting. Fish were transferred to clean aquaria about every 30 days. Samples of live fish were collected at 90-d intervals after exposure for external observations and histopathological examination. Five fish per replicate were randomly netted, anesthetized in tricaine methanesulfonate (Finquel®, Argent Chemical Co.), and preserved in Dietrich's fixative (29% ethanol, 10% formalin, and 2% glacial acetic acid in deionized water). Gross external examinations for fin erosion were made with a binocular dissecting microscope. Mortalities were noted during and after the exposure period.

Tissues were prepared for histopathological analyses by adapting routine procedures (Sheehan and Hrapchak 1980). Fixed specimens were dehydrated through the standard ethanol series, cleared with xylene, and infiltrated and embedded in paraplast. The specimens were then mounted longitudinally on a rectangular embedding cassette clamped in a microtome chuck. Paraffin blocks were trimmed with the microtome blade until the mid-sagittal region of the specimen was exposed. Mid-sagittal sections (10 to 15 per specimen, 5 μm thick) were cut and mounted on glass microslides, stained with Gill's hematoxylin, counterstained with Phloxine-Eosin, coverslipped, cleaned, and labeled. Microslides were prescreened for quality and for the presence of the desired organs and tissues (especially liver, pancreas, gut, spleen, gonad, swim bladder, kidney, heart, and brain). From each specimen, the three best microslides were selected for histopathologic evaluation. We designated basophilic, eosinophilic, and clear cell foci as incipient neoplasms. These neoplasms are small (less than 1.0 mm in diameter), well-differentiated, noncompressive, enzyme-altered clonal populations of clear or amphophilic hepatocytes representing an early stage of (if not actually committed to progressing to) an obvious neoplasm. Obvious neoplasms have, in addition to tinctorial changes, varying degrees of cytologic and pattern atypia (or both). Obvious neoplasms also have the possibility of compressive, invasive, and metastatic behavior and are often larger than 1.0 mm in diameter.

Data on frequency of fin erosion and liver abnormalities were analyzed with linear categorical models (Grizzle *et al.* 1969). The CATMOD procedure (SAS Institute 1987) was used to test for differences between extract treatment groups and controls. Because of the presence of zero frequencies, 0.1 was added to each raw frequency used in this analysis.

Results and Discussion

GC/MS Analysis of Acetone-Solubilized Crude Sediment Extracts

Polycyclic Aromatic Compounds (PACs): As reported by Fabacher *et al.* (1988), sediment extracts from the four contaminated sites contained various amounts of PAC contamination that reflected the general degree of contamination of the sediment and subtle differences in the composition of the organic fractions. Concentrations of PACs in sediments from Munuscong Lake, the reference site, were low; in contrast, the Black River contained both the highest concentrations and greatest variety of PACs and alkylated PACs. The A-2 fraction from the Black River contained 12 PACs, including known carcinogens, at concentrations of 1.0 μg/g or greater (e.g., benzo[*a*]pyrene) and others (benzo[*a*]fluorene, perylene and benzo[*g,h,i*]perylene) at 3.0 μg/g or greater. Fabacher *et al.* (1988) also reported that in contrast to sediment extracts from the other collection sites, those from the Black River contained quantifiable total N-PACs. Most abundant among these were carbazole and several substituted carbazoles.

With GC/MS, we were able to detect and quantitate many more PACs in the ARCEs than Fabacher *et al.* (1988) reported on the basis of GC/FID quantitation and GC/MS confirmation of alumina fractions 2 and 3 (Table 2), and included PAHs and PACs substituted with nitrogen, sulfur, or oxygen. In our study, the ARCE's from the four industrialized sites contained 5.6–313.3 μg total PACs/g sediment and 3.0–36.4 μg other compounds/g sediment. The ARCE from the reference site contained 1.55 μg total PACs/g sediment. We detected carbazole in all ARCEs except Munuscong Lake, whereas Fabacher *et al.* (1988) reported carbazole only in the A-3 fraction of the Black River. We also found perylene, indeno[1,2,3-*c,d*]pyrene, dibenz[*a,h*]anthracene, and benzo[*g,h,i*]perylene in the Fox River sediment extract at concentrations ≥0.88 μg/g; these compounds were not reported by Fabacher *et al.* (1988).

Many alkyl-substituted PACs were also found in the ARCEs (Table 2), which significantly increased the total PAC concentrations relative to the totals reported by Fabacher *et al.* (1988). For example, more than two-thirds of the PACs quantitated in the Fox River ARCE (228.7 μg/g sediment) were alkyl-substituted. Consequently, our PAC grand totals are highest for the Fox River (313.3 μg/g—Table 2). Although the contract laboratory that performed the analyses for Fabacher *et al.* (1988) acknowledged the presence of unresolved alkylated PACs, these compounds were

not reported, which resulted in a lower total for the Fox River (81.8 μg/g vs. 313.3 μg/g in our study). The predominance of unsubstituted PACs, many of which are EPA priority pollutants (National Resources Defense Council, Inc., *et al.* V. Train, 1976, 1979; Federal Register, 1984) confirms previous findings that most of the PACs in the Black and Cuyahoga Rivers were combustion-derived. In contrast, the alkyl-PACs, which predominated in extracts from the Fox River, typically result from non-combustion sources such as petroleum and other fossil fuels. The full extent of the PACs in extracts containing such compounds may not be accurately assessed by analysis for only EPA priority pollutants.

Despite the already noted differences between our analyses of ARCEs and previous analyses of alumina fractions (Fabacher *et al.* 1988) and the incomplete solubilization of the extracts by acetone (Table 1), some PAC concentrations were the same in both studies. For example, the concentrations of phenanthrene, fluoranthene, pyrene, and benz[*a*]-anthracene are in close agreement for the Cuyahoga River crude extracts and ARCEs. Concentrations of these four PACs differed slightly more in the Black River, Fox River and Munuscong Lake crude extracts and ARCEs, and differed considerably for the Menominee River crude extracts and ARCEs.

PCBs, Organochlorine Pesticides, and Other Compounds

The GC/MS analyses included detection and estimation of concentrations of PCBs (Table 3). Total concentrations of PCBs containing 2–5 chlorines were highest in ARCEs from the Fox River, totaling an estimated 0.8–3.2 μg/g, and were followed closely by the total of PCBs containing 3–6 chlorines in the ARCE from the Cuyahoga River, which totaled an estimated 0.3–1.2 μg/g. PCBs were present at levels below the limit of quantitation (<0.2 μg/g) in the Black River ARCE and were not detected (<0.2 μg/g) in either the Menominee River or Munuscong Lake ARCEs. In a separate study, total PCBs were detected by capillary GC with electron capture detection at 0.75–0.99 μg/g in sediment from the Fox River, 0.49 μg/g from the Cuyahoga River, and 0.04 μg/g from the Menominee River (U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, MO and National Fisheries Research Center-Great Lakes, Ann Arbor, MI, unpublished data).

Few organochlorine pesticides were detected by GC/MS. In the Cuyahoga River ARCE, *p,p'*-DDE and two isomers of chlordane were present at concentrations near the limit of quantitation (≤0.05 μg/g). No non-*o*-chloro-PCBs, polychlorinated dibenzofurans, or polychlorinated dioxins were detected by full-scan GC/MS. When a more sensitive and specific procedure was used (Smith *et al.* 1984), many compounds were detected in sediments from all four industrialized sites at concentrations of 0.01–40 ng/g (P. C. Baumann, U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center Field Station, Columbus, OH, 1990, unpublished data).

More of other compounds were identified and characterized by GC/MS in the Fox River ARCE than other sites (Table 4). These included several alkyl-substituted diphenylmethanes, which are PCB replacement compounds previously identified in sediments from the Fox River (Peterman and Delfino 1990). In contrast to PACs, most of which are metabolized by fishes to a large extent, these compounds bioaccumulate in fishes (Peterman and Delfino 1990). Detection of all three cresol (methyl phenol) isomers and acetophenone in the Fox River ARCE is probably related to the presence of paper mills (Wisconsin Department of Natural Resources 1978).

Among the other detected compounds (Table 4) were oxidized PACs such as quinones, ketones, and alcohols. Anthraceneone, anthracene-dione, benz[*a*]anthraceneone, and benz[*a*]anthracene-dione are photodegradation products of anthracene and benz[*a*]anthracene, respectively, two of the PACs most sensitive to photooxidation. Some of these oxygen-containing PACs (O-PACs) were identified in several ARCEs. Anthracene-dione was detected in the ARCEs of sediment from the three most contaminated rivers. The presence of anthracene-dione suggests that some oxidation of native anthracene occurred prior to analysis because D_{10}-anthracene was quantitatively recovered from the ARCEs. Ligocki and Pankow (1984) showed that D_{10}-anthracene in acetone photodegraded to D_8-anthracenedione. Other O-PACs tentatively identified in the Menominee River ARCE include fluorenol, fluoreneone, and phenanthrenol. We do not know whether these PAC-alcohol breakdown products were present prior to sediment collection or were formed later. Also, the lower than previously reported (Fabacher *et al.* 1988) PAC concentrations for the Menominee River ARCE suggest that some PACs degraded, which may occur at −20°C (Sverdrup *et al.* 1990).

Despite uncertainties, the GC/MS analyses identified and quantitated numerous PACs, many of which were alkyl-substituted. Although not identical, concentrations of some PACS in our GC/MS re-analyses were comparable to those reported by Fabacher *et al.* (1988). We also found PCBs and other previously unreported compounds in the ARCEs. The GC/MS analyses of the ARCEs also demonstrated that sediments from the four industrialized sites contained residues of carcinogenic chemicals and that these carcinogens were delivered to the test fish through the exposure protocol.

Toxicity and Carcinogenicity of Sediment Extracts

Toxicity: Mortality of medaka during the exposure period averaged 11% (range, 0–21%) in controls and carrier controls but was higher in many ARCE, ARA-2F, and ARA-3F exposures (Table 5). Mortality increased with dose level during the exposures to ARCEs and was high (15–45%) in fish exposed to all ARCEs except Munuscong Lake ARCE, which was much lower (1%). For the ARA-2Fs, exposure to 0.5 mg/L from Munuscong Lake produced mortality that was similar to controls, whereas mortality was greatest in fish exposed to 1–2 mg/L from the Black and Fox Rivers. Concentrations of ARA-2Fs causing mortality were much lower than those used in the ARCE exposures (Table 5). No ARA-3Fs caused considerably greater mortality than that which occurred in controls. Post-exposure mortality was similar in controls, MAMA exposures, and ARCE, ARA-2F, and ARA-3F exposures and was probably due to

Table 3. Estimated concentrations (μg/g sediment on a dry weight basis) of PCBs in acetone-redissolved crude extracts (ARCEs) from Great Lakes sediments. Number of congener peaks summed are listed in parentheses

Compound name	MW[a]	Collection site				
		Black R.	Cuyahoga R.	Fox R.	Menominee R.	Munuscong L.
Cl_2-PCBs	222	—[b]	—	0.26 (2)	—	—
Cl_3-PCBs	256	—	0.13 (2)	0.55 (3)	—	—
Cl_4-PCBs	290	<0.03[c] (1)	0.24 (6)	0.58 (8)	—	—
Cl_5-PCBs	324	<0.04 (1)	0.16 (6)	0.19 (6)	—	—
Cl_6-PCBs	358	<0.04 (1)	0.10 (3)	—	—	—
Cl_7- to Cl_{10}-PCBs[d]	392–494	—	—	—	—	—
Estimated total: Concentration Range[e]		<0.2 (5)	0.3–1.2 (17)	0.8–3.2 (20)	—	—

[a] Molecular weight
[b] Not detected
[c] Detected below the limit of quantitation
[d] Cl_7-PCBs, Cl_8-PCBs, Cl_9-PCBs and Cl_{10}-PCB were not detected in all samples. (Limits of Quantitation were 0.07, 0.07, 0.08, and 0.10 μg/g, respectively)
[e] Estimated total concentrations fall within ranges because quantitation did not include measured relative response factors from a comprehensive PCB standard mixture

overcrowding or poor water quality in holding tanks. No mortality occurred during the 24-h MAMA exposure (Table 5).

Fin Erosion and Non-Neoplastic Liver Abnormalities

Fin erosion was common in medaka exposed to extracts, but was generally rare in controls (Table 6). Frequencies of fin erosion and liver abnormalities in controls and carrier controls were not significantly different within ARCE, ARA-2F and ARA-3F exposures; the control frequencies were therefore combined for statistical comparisons. Caudal and pectoral fin erosion was most common in the ARCE exposures, with frequencies as high as 82% (Table 6). All ARCEs except Munuscong Lake produced significantly higher frequencies of caudal or pectoral fin erosion than controls, and the incidence of erosion for both increased significantly after exposure to Black, Cuyahoga, and Menominee River ARCEs. Fin erosion has also been reported to occur in fishes exposed to crude and waste transformer oils (Minchew and Yarbrough 1977; Woodward *et al.* 1981; Mayer *et al.* 1985). None of the ARA-2Fs or ARA-3Fs produced significant increases in fin erosion.

Hepatic lipidosis and spongiosis hepatis occurred in both control and treated medaka (Table 6). We considered as positive only moderate and severe cases of lipidosis, which consisted of large clusters or extensive areas of hepatocytes containing round, sharply defined cytoplasmic vacuoles that resembled the fatty livers of medaka experimentally exposed to diethylnitrosamine (DEN) (Hinton *et al.* 1988). Lipidosis frequency was increased significantly over controls by exposure to ARA-2Fs from all sites, and was also high in some ARCE and ARA-3F exposures, although these were not significantly different from controls (Table 6). ARCEs, ARA-2Fs, and ARA-3Fs from Fox and Black River sediments consistently produced high frequencies of lipidosis. Although mild lipidosis is a natural and reversible condition of well-fed fishes (Halver 1972), the severity and uneven distribution of lipidosis among treatments suggests a toxic influence of the extracts, especially the ARA-2Fs.

Spongiosis hepatis, an irregular, intrahepatic, multilocular formation of probable perisinusoidal cell origin (Bannasch *et al.* 1981), is a possible indicator of chemical exposure in fishes (Couch 1990). This condition, which occurred less frequently than lipidosis, was most prevalent in ARA-2F exposures, although it was also common in all Fox River exposures (Table 6). The frequency of spongiosis hepatis was not significantly different from controls in any ARCE, ARA-2F, or ARA-3F exposures, however (Table 6). Other investigators have reported similar findings. Bunton (1990) found spongiosis hepatis in medaka exposed to DEN and, at low incidence, in controls. When Maccubbin *et al.* (1987) treated medaka eggs with a topical application of benzo[*a*]pyrene (BP) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in DMSO (passive perchorionic exposure method), histopathological analysis at 4 months postexposure revealed spongiosis hepatis in about 60% of the fish that had received BP and about 50% of those exposed to MNNG. Controls had a low incidence (5%) of primarily the early stage of this lesion, and carrier (DMSO) controls had an incidence of about 24%. Maccubbin *et al.* (1987) observed only one tumor (a cholangioma) in medaka exposed to BP, and no definite neoplasms in response to river sediment extracts; however, medaka that had been exposed to Buffalo River sediment extract had spongiosis hepatis and eosinophilic foci of altered hepatocytes. In the same study, MNNG, BP and Black River sediment extract microinjected into the yolk sac of late eyed-

Table 4. Concentrations (μg/g sediment on a dry weight basis) of other compounds identified or characterized by GC/MS in acetone-redissolved crude extracts (ARCEs) from Great Lakes sediments

Compound name	MW[a]	SCAN[b]	ID[c]	Collection site				
				Black R.	Cuyahoga R.	Fox R.	Menominee R.	Munuscong L.
Cresol (methyl phenol)[d]	108	490	P[e]	—[f]	—	0.4[g]	—	—
Cresol (methyl phenol)	108	515	P	—	—	0.3	—	—
Acetophenone	120	535	P	—	—	2.	—	—
Cresol (methyl phenol)	108	540	P	—	—	3.	—	—
Methyl acetophenone or C_4-benzene	134	605	T[h]	—	—	0.3	—	—
Chloro-phenyl-isocyanate[i]	153	623	P	0.3	0.1	—	—	—
Camphor or other $C_{10}H_{16}O$	152	642	T	—	—	0.9	—	—
Methyl dihydro-indene or equivalent	132	645	P	—	—	0.4	—	—
Borneol or other $C_{10}H_{18}O$	154	668	T	—	—	0.8	—	—
Tetrahydro-2H-pyran-2-ol or related	U[j]	757	U[j]	—	—	10.	0.3	—
C_2-Dihydro-indene or equivalent	146	774	P	—	—	0.3	—	—
C_2-Dihydro-indene or equivalent	146	790	P	—	—	0.6	—	—
Methyl benzothiazole	149	830	P	—	—	—	1.	—
Dichloro-phenyl-isocyanate	187	842	P	0.3	0.2	0.1	—	—
C_2-Tetralin (C_2-tetrahydronaphthalene)	160	859	P	—	—	0.3	—	—
C_3-Tetralin	174	883	P	—	—	0.2	—	—
Tetrahydro-2H-pyran-2-ol or related	U	888	U	—	—	2.	0.1	—
C_3-Tetralin	174	905	P	0.1	—	0.2	—	—
C_3-Tetralin	174	945	P	—	—	0.2	—	—
C_2-Tetralin or equivalent	160	947	T	—	—	1.	—	—
C_3-Tetralin	174	981	T	—	—	0.2	—	—
Biphenylol or diphenyl ether	170	1056	T	0.2	0.1	1.	—	—
Aromatic compound	153	1084	U	—	—	0.4	—	—
Alkyl-substituted aromatic compound	188	1093	U	—	—	2.	—	—
Pyrido[ ][k]pyrimidin-one	147	1130	T	—	0.2	—	—	—
C_8-Phenol	206	1178	T	—	—	2.	—	—
Methyl thio-benzothiazole	181	1178	T	—	—	—	<0.1[l]	—
Naphthalenyl-ethanone	170	1190	P	—	—	—	<0.1	—
Phenalene or similar PAH	166	1204	T	1.	—	—	—	—
2-Ethyl diphenylmethane[m]	196	1211	T	—	—	0.5	—	—
Xanthene or methyl dibenzofuran	182	1225	P	0.1	0.1	—	—	—
Benzothiazolone	151	1241	P	—	—	0.3	—	—
4-Ethyl diphenylmethane[m]	196	1244	T	—	—	1.	—	—
Hexenyl naphthalene or equivalent	210	1258	T	—	0.3	—	—	—
Aromatic compound	180	1258	U	0.3	—	—	—	—
Hexathiepane (CH_2S_6)	206	1301	P	—	—	—	<0.1	—
Fluorenol	182	1317	P	—	—	—	0.2	—
Alkyl-substituted compound	U	1346	U	—	—	—	1.	—
Naphtho[ ]thiophene or equivalent	184	1387	T	0.2	—	—	—	—
Phenanthrenol or equivalent	194	1399	T	—	—	—	<0.1	—
$C_{16}H_{14}$	206	1420	U	—	—	0.5	—	—
Aromatic compound	204	1434	U	0.9	—	—	—	—
Alkyl-substituted aromatic compound	U	1442	U	—	—	1.	—	—
Anthracenone, Phenanthrenol or other	194	1460	U	—	—	—	<0.1	—
Fluorenone or equivalent	180	1473	T	—	—	—	<0.1	—
o-Terphenyl	230	1495	P	—	—	—	<0.1	—
Alkyl-substituted aromatic compound	208	1501	U	0.1	0.1	—	—	—
Alkyl-substituted aromatic compound	208	1507	U	0.2	0.1	—	—	—
Alkyl-substituted aromatic compound	224	1510	U	0.2	0.1	—	—	—
D_8-Anthracene-dione[n]	216	1533	T	—	—	—	0.1	0.2
Anthracenedione[o]	208	1542	P	0.6	0.6	0.3	—	—
Alkyl-substituted aromatic compound	224	1550	U	0.2	—	—	—	—
Phenyl indene-dione or equivalent	222	1629	T	—	—	—	<0.1	—
Alkyl-substituted aromatic compound	260	1640	U	—	—	—	2.	—
Tetrahydro-retene[p] or other $C_{18}H_{22}$	238	1662	T	—	0.3	0.8	—	—
Aromatic compound	208	1663	U	0.6	—	0.5	—	—
Aromatic compound	U	1670	U	—	—	0.3	—	—
Aromatic compound	222	1757	U	—	—	0.3	—	—
Retene[p]	234	1770	T	0.2	0.1	1.	<0.1	—
Aromatic compound ($C_{18}H_{16}$)	232	1803	U	—	0.1	0.5	—	—

(Continued on next page)

Table 4. (cont'd.)

Compound name	MW[a]	SCAN[b]	ID[c]	Collection site				
				Black R.	Cuyahoga R.	Fox R.	Menominee R.	Munuscong L.
Aromatic compound	218	1811	U	—	—	0.1	—	—
Aromatic compound	232	1837	U	—	—	—	<0.1	—
Alkyl-substituted aromatic compound	252	1840	U	—	—	—	0.1	—
Benz[ ]anthracene-one	230	1902	T	—	—	—	0.1	—
Dioctyl Adipate	370	1926	P	—	—	—	3.	—
Benz[ ]anthracene-one	230	1958	T	—	0.2	—	0.1	—
Benz[*de*]anthracen-7-one	230	2040	T	0.4[q]	—	—	0.3	—
D_{10}-Benz[*a*]anthracene-dione[r]	268	2118	T	—	—	—	—	0.1
Benz[*a*]anthracene-dione[s]	258	2127	T	—	0.1	—	<0.1	—
Aromatic compound	254	2145	U	—	0.1	—	—	—
Nitrogen-containing PAC[t]	245	2237	U	—	—	0.1	—	—
PAC, possibly $C_{18}H_{14}N_2$	258	2295	U	—	—	0.2	—	—
PAC, possibly $C_{18}H_{14}N_2$	258	2330	U	—	—	0.2	—	—
PAC	268	2340	U	0.2	0.1	—	—	—
PAC, possibly $C_{18}H_{14}N_2$	258	2344	U	—	—	0.2	—	—
Aromatic compound	254	2358	U	—	—	—	0.1	—
a PAH	264	2483	U	0.2[u]	0.1	—	—	—
Total:				6.3	3.0	36.4	9.4	—
Number of compounds				(19)	(18)	(40)	(23)	

[a] Molecular weight
[b] Scan number of GC/MS acquisition, equivalent to GC/MS retention time in seconds
[c] Identification
[d] Compounds with the same name are different isomers detected at different scan numbers
[e] Probable identification by MS NIST Library match
[f] Not detected
[g] Compounds were quantitated to one significant place
[h] Tentative identification by MS interpretation
[i] Chem Abstracts #104-12-1, 3320-83-0, 2909-38-8, or 51134-03-3. Although it is a chemical intermediate, it may also result from a GC breakdown of trichlorocarbanilide (Jungclaus et al. 1978)
[j] Unknown
[k] Unspecified isomer
[l] Detected below the limit of quantitation
[m] A major constituent of Santosol[R] 100, a PCB replacement dye solvent mixture used in carbonless copy paper (Peterman and Delfino, 1990)
[n] A photolytic oxidation product of the internal standard, D_{10}-anthracene, not included in the total at the end of the table
[o] A photolytic oxidation product of anthracene
[p] Wakeham et al. (1980) found certain PAHs in lake sediments which could not be attributed to anthropogenic sources
[q] Reported in prior study at 1.8
[r] A photolytic oxidation product of the internal standard, D_{12}-benz[*a*]anthracene, not included in the total at the end of the table
[s] A photolytic oxidation product of benz[*a*]anthracene
[t] Polycyclic aromatic compound
[u] For the Black R. sample, the prior study reported an unknown MW 264 PAH as possibly cyclopenta[ghi]perylenes

stage rainbow trout eggs induced liver neoplasms, but not spongiosis hepatis.

Neoplasms

MAMA: Cholangioma was detected in three MAMA-exposed medaka at Day 90 (Table 7). One of these fish also had a basophilic focus (0.4-mm dia.), and one had a hepatocellular carcinoma. At Day 180, nine MAMA-exposed fish had one or more incipient neoplasms or neoplasms; one had a cholangioma and also a hemangiopericytoma between the liver/cholangioma and the abdominal skin, one a cholangioma and a small focus of basophilic hepatocellular alteration, one a 0.6-mm dia. focus of basophilic hepatocellular alteration, and two others had both hepatocellular carcinoma and cholangioma. Cholangioma was also present in three other fish, and one fish had a perisinusoidal cell neoplasm. A variety of neoplasms were also present in MAMA-treated fish at Day 270. Three fish each had a hepatocellular carcinoma; in one of these, the normal liver showed toxic changes in the form of hepatic lipidosis and numerous areas of spongiosis hepatis. This fish also had a hemangiopericytoma of the intestine. Another fish had a leiomyosarcoma of the gut wall, and two others each had a basophilic focus of hepatocellular alteration. One fish had two tinctorially different foci of hepatocellular alteration; one was basophilic (0.5-mm in dia.) and the other was eosinophilic (0.6-mm in dia.). One fish had both a 0.8-mm dia. clear cell focus and a 1.0-mm dia. basophilic focus of hepatocellular alteration. At Day 360, each of two MAMA-exposed fish had a poorly to moderately differentiated cholangioma.

Table 5. Summary of dosages, extract and fraction concentrations, and mortality in medaka exposures. Mean percent mortality during pulse-dose period (28 days) and post-exposure period (270 days for groups I–III; 360 days for groups IV and V) is adjusted for samples collected for histopathology

Exposure and dose	Concentration[f] (mg/L)	Mortality (%)	
		Exposure	Post-exposure
Exposure I			
Control[a]	—	15	50
Carrier control[b]	4.0[g]	21	53
Black R. ARCE[c], low	2.8	15	57
Black R. ARCE, medium	8.3	35	40
Black R. ARCE, high	22.0	42	47
Exposure II			
Control	—	0	47
Carrier control	6.0	5	69
MAMA	2.0	0	63
Black R. ARCE, extra-high	33.0	40	46
Fox R. ARCE, low	11.0	15	57
Fox R. ARCE, high	22.0	38	50
Munuscong L. ARCE, low	5.0	1	46
Exposure III			
Control	—	12	31
Carrier control	8.0	7	34
Cuyahoga R. ARCE, low	22.0	17	36
Cuyahoga R. ARCE, high	40.0	45	25
Menominee R. ARCE, low	11.0	22	30
Menominee R. ARCE, high	40.0	41	41
Exposure IV			
Control	—	9	66
Carrier control	0.5	13	53
Black R., ARA-2F[d] fraction	1.0	33	50
Cuyahoga R., ARA-2F fraction	2.0	24	53
Fox R., ARA-2F fraction	2.0	31	62
Menominee R., ARA-2F fraction	2.0	25	51
Munuscong L., ARA-2F fraction	0.5	7	59
Exposure V			
Control	—	14	52
Carrier control	1.25	12	48
Black R., ARA-3F[e] fraction	5.0	15	52
Cuyahoga R., ARA-3F fraction	5.0	14	39
Fox R., ARA-3F fraction	5.0	21	50
Menominee R., ARA-3F fraction	5.0	10	44

[a] Control fish in water alone
[b] Carrier was acetone
[c] Acetone-redissolved crude extract
[d] Acetone-redissolved A-2 fraction
[e] Acetone-redissolved A-3 fraction
[f] To calculate the ARCE water concentration (ng/L) of any compound listed in Tables 2–4, multiply its value in the table by the following conversion factors: Black R., low (0.28), medium (0.84), high (2.2), extra-high (3.4); Fox R., low (0.60), high (1.2); Munuscong L., low (6.1); Cuyahoga R., low (2.6), high (4.8); Menominee R., low (2.2), high (8.0)
[g] Carrier concentration expressed as mL/L

Extracts and Fractions: All fish examined at Day 90 were essentially normal; there were no proliferative, parasitic, or infectious lesions. Of the fish sampled and examined after exposure to ARCEs, ARA-2Fs, and ARA-3Fs at Day 180, one fish from the Black River ARCE high dose had a eosinophilic focus of hepatocellular alteration; one treated with the high dose of Fox River ARCE had a hepatocellular adenoma; one from the Black River ARA-2F exposure had a subtle, 0.15-mm dia. eosinophilic focus of hepatocellular alteration; one from the Cuyahoga River ARA-3F exposure had a well-differentiated 0.15-mm dia. eosinophilic focus of hepatocellular alteration; one from the Fox River ARA-3F exposure had a cholangioma; and one carrier control fish had two eosinophilic foci of hepatocellular alteration (≤0.5 mm dia.). Among the medaka exposed to extracts and fractions and examined at Day 270, one fish from the Cuyahoga River ARA-2F exposure had a 0.3-mm dia. eosinophilic focus of hepatocellular alteration that contained no mitoses but was lightly compressive. One fish from the Fox River ARA-2F exposure had a 0.5-mm dia. eosinophilic focus of hepatocellular alteration with no compression, no mitoses, and no lipid in the focus. One fish exposed to the high-dose Black River ARCE had a hepatocellular carcinoma characterized by a well-differentiated 3.5-mm dia. basophilic mass; this fish also had a 0.5-mm dia. basophilic focus to which normal liver conformed and which contained mitotic figures. Another fish exposed to the high dose of Black River ARCE had a hepatocellular carcinoma that appeared as two small lesions (0.6-mm dia. each). These lesions were characterized by hepatocytomegaly of two and three magnitude, a solid pattern, and a distinct, irregular, non-compressive boundary with normal liver; this was more atypia than simple alteration. One carrier control fish had two eosinophilic foci of hepatocellular alteration of 0.2- and 0.3-mm dia. Among the fish examined at Day 360, one from the Fox River ARA-3F exposure had a pancreatic duct cell adenoma that resembled the lesions reported by Thiyagarajah and Grizzle (1986) in *Rivulus* exposed to DEN. One fish from the control group had a disseminated lymphocytic lymphoma, a spontaneous lesion not attributable to chemical induction (Okihiro and

Table 6. Mean percentage of fin erosion and non-neoplastic liver abnormalities in medaka collected for histopathological analysis during the post-exposure period (days 90, 180, 270, 360)

Exposure	Type of abnormality			
	Caudal fin erosion	Pectoral fin erosion	Hepatic lipidosis	Spongiosis hepatis
MAMA[a]	0	0	50	27
ARCE[bc]				
Control[d]	0	0	13	1
Carrier control[e]	0	0	15	1
Black R.	78[h]	67[h]	24	0
Cuyahoga R.	82[h]	62[h]	8	0
Fox R.	13	62[h]	32	12
Menominee R.	69[h]	47[h]	0	0
Munuscong L.	0	0	5	0
ARA-2F[f]				
Control	3	0	0	15
Carrier control	2	0	13	7
Black R.	5	5	30[h]	13
Cuyahoga R.	2	0	33[h]	20
Fox R.	20	5	20[h]	10
Menominee R.	0	0	30[h]	13
Munuscong L.	2	0	23[h]	0
ARA-3F[g]				
Control	0	2	3	3
Carrier control	2	2	13	0
Black R.	5	5	17	3
Cuyahoga R.	2	2	3	0
Fox R.	0	0	7	13
Menominee R.	0	0	0	7
Munuscong L.	—[i]	—	—	—

[a] Methylazoxymethanol acetate
[b] Acetone-redissolved crude extract
[c] High dose levels of crude extracts were used for analysis
[d] Fish in water alone
[e] Fish in water and acetone
[f] Acetone-redissolved A-2 fraction
[g] Acetone-redissolved A-3 fraction
[h] Significantly different from controls and carrier controls at ($P \leq 0.05$)
[i] No A-3 fraction was produced in fractionation

Hinton 1989, Harada *et al.* 1990, Battalora *et al.* 1990). This lymphoma was the only spontaneous lesion observed among the 270 control fish examined (representing 0.37%).

In the carrier controls there were four incipient neoplasms (two in each of two fish) among 285 fish examined (representing 0.7%). These percentages of neoplasms and incipient neoplasms in controls are within the range reported elsewhere for spontaneous lesions in medaka (Hawkins *et al.* 1988; Masahito *et al.* 1989). There were no apparent neoplasms in medaka that died during the exposure or post-exposure period.

Relation to Previous Studies: Fabacher *et al.* (1988) reported that the crude extracts and A-2 and A-3 fractions from all the contaminated industrial sites were mutagenic in the Chinese hamster ovary hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) assay for mutagenicity. Munuscong Lake had a dose-response relation (slope) that was marginally significant ($P < 0.05$) for the A-2 fraction, but the responses were not significantly different from controls. The Cuyahoga River sediment extracts were the most mutagenic in both the CHO/HGPRT and rat hepatocyte unscheduled DNA synthesis assays. In our study, however, there were no neoplasms in medaka exposed to ARCE, ARA-2F, or ARA-3F from either Munuscong Lake or the Cuyahoga River. Our results further corroborate recent reports that *in vitro* assays for mutagenicity may not be as predictive of *in vivo* chemical carcinogenicity as had been previously expected (Tennant *et al.* 1987; Heddle 1988). Neoplasms induced by ARCEs from the Black and Fox rivers and ARA-3F from the Fox River included a hepatocellular adenoma, a cholangioma, and several cases of hepatocellular carcinoma. These kinds of neoplasms have also been detected in wild fishes inhabiting the Black River and other PAC-contaminated sites (Baumann *et al.* 1987; Baumann 1989). Fabacher *et al.* (1988) reported the presence of numerous carcinogenic PACs in sediments from these sites. Using GC/MS, we also detected components such as dibenzofluorenes and alkyl carbazoles in greater concentration in the Fox River than in the Black River ARCE (Table 2) that were not reported by Fabacher *et al.* (1988). Research by other investigators (Griest *et al.* 1979; Severson *et al.* 1978) suggests that multialkylated PACs with more than three aromatic rings contribute to the mutagenicity and tumor-initiating activity of complex PAC mixtures. Components such

Table 7. Incipient neoplasms and neoplasms in tissues of control and exposed medaka

Days post-exposure and exposure	Lesion
Day 90	
MAMA[a]	Cholangioma (3/15)[g]
	Hepatocellular alteration, basophilic focus[h] (1/15)
	Hepatocellular carcinoma (1/15)
Day 180	
MAMA	Cholangioma (7/15)
	Hemangiopericytoma (1/15)
	Hepatocellular carcinoma (2/15)
	Hepatocellular alteration, basophilic focus[h] (2/15)
	Perisinusoidal cell neoplasm (1/15)
Carrier Control[b]	Hepatocellular alteration, eosinophilic focus[h] (2/45)
Black R. ARCE[c], high	Hepatocellular alteration, eosinophilic focus[h] (1/15)
Fox R. ARCE, high	Hepatocellular adenoma (1/15)
Black R., ARA-2F[d]	Hepatocellular alteration, eosinophilic focus[h] (1/15)
Cuyahoga R., ARA-3F[e]	Hepatocellular alteration, eosinophilic focus[h] (1/15)
Fox R., ARA-3F	Cholangioma (1/15)
Day 270	
MAMA	Hemangiopericytoma (1/15)
	Hepatocellular carcinoma (3/15)
	Hepatocellular alteration, basophilic focus[h] (4/15)
	Hepatocellular alteration, clear cell focus[h] (1/15)
	Hepatocellular alteration, eosinophilic focus[h] (1/15)
	Leiomyosarcoma, intestine (1/15)
Carrier Control	Hepatocellular alteration, eosinophilic focus[h] (2/45)
Black R. ARCE, high	Hepatocellular alteration, basophilic focus[h] (1/15)
	Hepatocellular carcinoma (2/15)
Cuyahoga R., ARA-2F	Hepatocellular alteration, eosinophilic focus[h] (1/15)
Fox R., ARA-2F	Hepatocellular alteration, eosinophilic focus[h] (1/15)
Day 360	
MAMA	Cholangioma (2/7)
Control[f]	Lymphoma (1/20)
Fox R., ARA-3F	Pancreatic duct-cell adenoma (1/15)

[a] Methylazoxymethanol acetate treated fish
[b] Carrier Control fish in water and acetone
[c] Acetone-redissolved crude extract
[d] Acetone-redissolved A-2 fraction
[e] Acetone-redissolved A-3 fraction
[f] Control fish in water alone
[g] (Fish with lesions/number of fish in exposure group examined histopathologically.) Some fish had more than one lesion and more than one type of lesion
[h] Incipient neoplasm

as these in the Fox River ARA-3F could, at least in part, have caused the neoplasms in medaka exposed to this fraction. The low frequency of neoplasms produced by our exposure protocol precluded rigorous statistical analysis or more detailed comparisons with previous findings.

A literature search provided no references to any study where multiple pulse exposures of sediment extracts and fractions had been done with medaka. In a study with a single compound, Hawkins *et al.* (1988) exposed medaka to two 6-h exposures of water-borne benzo[*a*]pyrene. The first exposure was conducted on 6–10-day old medaka; the second exposure occurred 7 days later. With the high dose of 269 μg/L for the first exposure and 253 μg/L for the second, 26 of 73 fish examined (36%) had hepatocellular neoplasms 36-wk post-exposure that included 20 fish with hepatocellular adenomas and 6 with hepatocellular carcinomas. In our study, the most hepatic neoplasms (2 of 15 fish examined, 13%) occurred in fish exposed to the high-dose of Black R. ARCE. Because we had to dilute the sediment extracts and fractions so that 50% or more of the fish survived the acute toxicity of the dosages (Table 5), the concentration of individual compounds in the exposures was greatly reduced. For example, using the conversion factor (Table 5, Footnote f) for the high-dose of Black R. ARCE (2.2), the concentration of benzo[*a*]pyrene in the exposure was about 10 ng/L (0.01 μg/L)—considerably less than the concentration in the study by Hawkins et al. (1988). The total PAC and other compounds in the high-dose of Black R. ARCE was only about 456 ng/L (0.456 μg/L). Although we used four 24-h pulses and Hawkins *et al.* (1988) used two 6-h pulses, the high-dose of Black R. ARCE was remarkably potent in producing neoplasms in medaka. The high-dose of Fox R. ARCE and the Fox R. ARA-3F were also very potent; however, a review of Tables 2–4 discloses no pattern as to what component or components might be responsible for this potency, either singly or in combination.

Presence and Significance of Tumor Promoters

Neoplasia in fish liver may be perceived as a multistage process involving initiation, promotion, and progression (Hinton 1989). Feral fishes may experience initiation as embryos, juveniles, or adults by exposure to initiating types of chemical contaminants (*i.e.*, those which interact with the genome) in food, sediments, the water column, or more probably, a combination of these. Promotion of neoplasms may then result by exposure of the previously initiated fishes to promoting types of chemical contaminants, which cause cell proliferation. Chemicals from both biogenic and anthropogenic sources can be classified as initiators, promoters, or both (*i.e.*, complete carcinogens). For example, Nunez *et al.* (1989) found that dietary 17 β-estradiol was an effective promoter of hepatocarcinogenesis in aflatoxin-B1 exposed rainbow trout. These authors suggested that their observations were particularly relevant to neoplasms in fishes from polluted environments because such neoplasms may be a result of weak carcinogens (initiators) acting in combination with other contaminants that may promote the carcinogenic response. Similarly, Hinton (1989) suggested that when fishes previously initiated by carcinogens are ex-

posed to promoter compounds within sediments or food, promotion of carcinogenesis is probable. Carbazole and Aroclor® 1254 enhanced the tumor response in rainbow trout when administered in the diet either before fry exposure or after embryo exposure to the initiator 7,12-dimethylbenz[*a*]anthracene (Hendricks, J. D., Department of Food Science and Technology, Oregon State University, Corvallis, Oregon, personal communication, 1990). Thus, the presence and concentration of potential promoter compounds such as PCBs, carbazole, and alkyl-substituted diphenylmethanes in the ARCEs, ARA-2Fs, and ARA-3Fs we tested may have influenced the number of tumors induced in the medaka.

Neoplasms in Fishes and Their Relevance to Environmental Quality

Mix (1986) concluded that although epizootics of tumors and neoplasms have been described in fish populations from many areas throughout the world, the evidence was not sufficient to prove that environmental pollutants are the universal cause. In review, Mix concluded that experimental data implicating chemical contaminants in the environment for cancer-like conditions in fishes from some areas of Puget Sound (Washington), the Fox River (Illinois), and Japan were adequate whereas the data purportedly supporting a chemical cause for highly publicized epizootics of tumors in fishes from the Buffalo River (Black 1982), Torch Lake (Black *et al.* 1982), and the Black River (Baumann *et al.* 1982) were not of high quality. Mix further concluded that most of the latter studies were compromised by inadequate experimental designs and that additional research, particularly laboratory studies, are necessary to document any role of chemical contaminants in these epizootics. In contrast, Metcalfe (1990) states in the preface of a special issue of *The Science of the Total Environment* comprising articles addressing chemical contaminants and fish tumors, that "Polycyclic aromatic hydrocarbons (PAHs) are almost certainly involved in the development of tumors in feral fish populations inhabiting contaminated marine and freshwater ecosystems." Although the results of our study do not prove that contaminants in sediments from Great Lakes tributaries cause tumors in brown bullheads, the data support the hypothesis that contaminants are a causal factor for tumors in fishes from the Black River. The neoplasms induced in medaka exposed to sediment extracts and fractions in our study were considerably fewer than expected based on field observations of the high incidence of neoplasms in brown bullheads from the test sites. Nevertheless, we suggest that our findings, particularly those from the Fox and Black rivers, and reports of others (Maccubbin *et al.* 1987; Metcalfe *et al.* 1988; Metcalfe 1990) add further credence to a chemical etiology for neoplasia in fishes associated with contaminated sediments.

Conclusions and Recommendations

Investigators should be aware that sediment extracts may contain substantial amounts of alkyl-PACs that may not be detected or reported when analyzing for only typical EPA priority pollutant PACs, and that the presence of alkyl-PACs may alter the effects of the extracts in assays for toxicity, mutagenicity, or carcinogenicity. Our results and those of Hinton *et al.* (1988) and others suggest that the effects of chemical carcinogens on medaka can be either non-carcinogenic or carcinogenic. Non-carcinogenic responses include spongiosis hepatis, hepatic lipidosis, and acute cytotoxicity; carcinogenic responses include incipient and complete neoplasms. In our study, the sediment extracts and fractions from many contaminated sites were non-carcinogenic in medaka, but the Black River ARCE and Fox River ARA-3F induced carcinomas in some fish of types normally associated with chemical exposure in wild fishes, further substantiating the hypothesis that contaminants in sediments are involved in epizootics of neoplasms in wild fishes at chemically contaminated sites.

We suggest that if investigators consider the multiple pulse method of dosing medaka with sediment extracts and fractions, more than four multiple pulse doses should be used to help ensure that any promoters present are able to exert their effects. It is also recommended that investigators consider adding an initiator and promoter to different aliquots of sediment extracts or fractions to be tested for carcinogenicity. The separate addition of an initiator or promoter may clarify any undetermined carcinogenic potential because if one or the other is lacking in the extract or fraction, the process of carcinogenesis may not proceed to completion.

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