CONTAMINANT LEVELS IN FISH AND SHELLFISH FROM THE EMAP-ESTUARIES LOUISIANIAN PROVINCE

[1,2]W.H. Benson, [1,2]J.M. O'Neal, and [2]J.C. Allgood
[1]Department of Pharmacology and [2]Environmental Toxicology Research Program, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi

and

J.K. Summers
U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida

INTRODUCTION

The Environmental Monitoring and Assessment Program (EMAP) is a national program developed by the U.S. Environmental Protection Agency in response to the need for information about the degree to which existing pollution control programs and policies protect the nation's ecological resources. EMAP - Estuaries represents one portion of EMAP's efforts in near coastal environments. These efforts are designed to provide a quantitative assessment of the regional extent of coastal environmental problems by measuring status and change in selected condition indicators. The Louisianian Province Demonstration Project, which focuses on the Gulf of Mexico, provides a mechanism by which cooperators can collect and assemble environmental data relevant to the Gulf.

Currently, one-sixth of the U.S. population lives in states bordering the Gulf of Mexico. Many of these citizens either directly or indirectly depend on the Gulf of Mexico for their livelihood (DOC 1990a; 1990b). Two-thirds of the contiguous U.S. drains into the Gulf of Mexico (Buff and Turner 1987). Ports along the Gulf handle 45% of U.S. import-export shipping tonnage. Approximately one-third of the marine recreational fishing activities in the continental U.S. occur in the Gulf. Forty percent of the U.S. commercial fish and shellfish yield, approximately 2.5 billion pounds each year, comes from the Gulf. The Gulf provides critical habitat for 75% of the nation's migrating waterfowl, some 500 species, and is home to numerous endangered species (EPA 1992). Nevertheless, to date, relatively little attention has been focused on environmental concerns in the Gulf as compared to its counterparts in the northwest and northeast.

The purpose of this investigation was to address these items of concern by conducting analytical chemistry evaluations which encompassed species (fish and shellfish) collected from the Gulf of Mexico. Specifically, this study represents the second year of data available for analytical chemistry evaluations from the EMAP - Estuaries Project for 1993.

MATERIALS AND METHODS

Sample Collection and Preparation

Sampling was conducted during the summer of 1993 (July to September). An unbiased sampling design was used so that major estuarine resources were sampled proportionately. This sampling design makes it possible to estimate the proportion or amount of area in the Louisianian Province having defined environmental conditions. Specifics of the sampling design for the 1993 Louisianian Province Demonstration are documented in Summers et al. (1991) while specifics related to the conduct of field sampling can be found in Summers et al. (1992). Briefly, 150 sites between Anclote Anchorage, Florida, and the Rio Grande, Texas, were sampled by trawling during the collection period. Species collected for chemical analysis included Atlantic croaker (*Micropogonias undulatus*), hardhead catfish (*Ariusfelis*), gafftopsail catfish (*Bagre marinus*), blue catfish (*Ictalurus furcatus*), brown shrimp (*Panaeus aztecus*), and white shrimp (*Panaeus setiferus*). Up to ten individuals were retained from each collection site. The specimens were sorted by species,

labeled, frozen on dry ice, packaged, and shipped to the Environmental Toxicology Research Program (ETRP) Analytical Laboratory for subsequent analysis for the contaminants listed in Table 1.

Samples were received by the ETRP Analytical Laboratory within 24 hr of collection. Upon receipt, samples were inspected, logged-in to the laboratory sample tracking system, and stored (-20°C) until further use. Specimens were partially thawed prior to removal of fillets. For scaled species, scales were removed prior to preparation of fillets. For non-scaled species (catfish), the skin was not taken as part of the filet. Each sample (1 to 10 specimens) from a collection site was filleted using a ceramic blade knife to avoid target metals contamination. The fillets from a given sample were composited to yield a homogeneous sample. The composite sample was placed in a labelled, glass, wide mouth bottle, sealed with a teflon-lined closure, and returned to the freezer until extraction and metals digestion procedures.

Moisture Determination. A sample (usually one gm) of tissue was weighed and lyophilized for 12 hr in a freeze dryer (Virtis). After 12 hr, the sample was again weighed and the percentage of moisture was determined. Previous experiments had shown that 12 hr in the freeze dryer was sufficient to reach a constant weight.

Extraction/Digestion and Sample Cleanup

Organics. All reagents used were of pesticide grade. Ten grams of tissue were spiked with internal standards (PCB congeners 103 and 198). The tissue was then sonicated (Tekmar TSD-600) for 1 min with 10 ml of acetonitrile (Fisher) in a 50 ml centrifuge tube and centrifuged for 5 min. The acetonitrile was removed and reserved. This procedure was repeated for a total of three times, thereby yielding 30 ml of acetonitrile extract per sample. Each of the three 10 ml acetonitrile extracts were added to the same 1 L separatory funnel containing 70 ml of pentane-washed, de-ionized (DI) water containing 2% sodium sulfate. The acetonitrile-sodium sulfate solution was triple extracted using 10 ml of pentane. The resulting 30 ml pentane extract was placed in a 250 ml beaker containing 10 gm of anhydrous sodium sulfate for water removal. The dried pentane extract was quantitatively transferred to a 125 ml flask and the volume reduced to approximately 3 ml by nitrogen evaporated solvent reduction (Organomation N-Evap).

A 200 mm x 9 mm ID liquid chromatography column with reservoir (Supelco, 280 mm overall) was packed with 3.5 gm of Florisil (Supelco, 60/100 mesh, activated at 130°C for 24 hr) and topped with 1.5 gm of anhydrous sodium sulfate (Fisher). The column was then washed with 20 ml of hexane (Fisher). When the hexane had nearly reached the top of the sodium sulfate, 3 ml of tissue extract was quantitatively transferred to the column. The column was then eluted first with 20 ml of 5% ethyl ether (Fisher) in hexane followed by 20 ml of 10% ethyl ether in hexane. The respective fractions were combined and then reduced to slightly less than 1 ml using an N-Evap and 25 ml concentrator tubes (Kontes). At this point, a postextraction-preinjection spike of tetrachlorometaxylene (TCMX) (Accustandard) was added to the extract to aid in monitoring the performance of gas chromatography (GC). The volume level of the extract was returned to a final volume of 1 ml. The extract was then transferred to a GC autosampler vial and sealed with a crimp top closure containing a Teflon-lined septum.

Heavy Metals. Samples were prepared using microwave digestion (CEM Model MDS-2100). Three to five gm of tissue sample were treated with 10 ml of concentrated nitric acid (J.T. Baker Instra-Analyzed) and 1 ml of concentrated HCl (Fisher). The digest were allowed to cool and volumetrically diluted to a final volume of 100 ml.

Instrumental Analysis

The required method detection limits (MDLs) for organics (except toxaphene) were 2 ng/g, dry weight. The MDL for toxaphene was 500 ng/g, dry weight. The required MDLs for heavy metals in units of µg/g, dry weight were: aluminum, 10.0; arsenic, 2.0; cadmium, 0.2; chromium, 0.1; copper, 5.0; lead, 0.1; mercury, 0.01; nickel, 0.5; selenium, 1.0: silver, 0.01; tin, 0.05; and zinc, 50.0.

Gas Chromatography. All analyses for organics were performed using a Hewlett-Packard Model 5890 Series II capillary GC equipped with dual autosamplers and dual H-P standard 63-Ni electron capture detectors and associated electronics. Splitless injection was used. The fused silica capillary column used for each channel of the GC was a 60 m, 0.25 mm ID, 0.25 micron film thickness DB-5 (J&W Scientific). Helium was the carrier gas with a linear velocity of 40 cm/s. The injector temperature was 280°C and the detector temperature was 310°C. Temperature programming was used to chromatograph the samples: an initial oven temperature of 150°C was held for 40 min, raised to 195°C at 1°C/min, held at 195°C for 20 min, raised to 220°C at 1°C/min, held at 220°C for 2 min, raised to 280°C at 3°C/min, and finally held there for 17 min. A flow of 2 ml/min was used to sweep the septum. The detector makeup gas was nitrogen at a flow rate (including carrier gas) of 60 ml/min. Chromatographic data were collected on a Hewlett-Packard Vectra 386/25 data station using Hewlett-Packard Chemstation software. Quantitation was by the method of internal standards (PCBs 103 and

198). A three point calibration curve also was established for backup purposes.

Atomic Absorption Spectrophotometry. A Varian Spectra 20 flame atomic absorption (AA) spectrophotometer was used to analyze for aluminum, copper, and zinc. An air/acetylene flame was used for copper and zinc. A nitrous oxide/acetylene flame was used for aluminum. The Varian Spectra 20 also was used for the analysis of mercury by the cold vapor method. The Varian VGA-76 Vapor Generation Accessory was used for this flameless method. Arsenic, cadmium, chromium, lead, nickel, selenium, silver, and tin were determined by use of a Varian Spectra 400Z graphite system with the Zeeman background correction system and the autosampler. Platforms were not used in the graphite tubes. Photron Super Lamps were used for arsenic and selenium determinations. For all metal analyses, regardless of technique, a three point calibration curve plus blank was performed.

Quality Assurance/Quality Control

Samples were analyzed in batches of 10. For each batch, a three point calibration curve plus blank was established. This curve was verified by an initial calibration verification sample prior to analysis. Other quality assurance/quality control (QA/QC) samples were: a laboratory reagent blank, a laboratory control material or a standard reference material, a duplicate, a matrix spike, and a continuing calibration verification sample at the end of the run.

SUMMARY OF FINDINGS

An overview of contaminant levels observed in the edible flesh of Atlantic croaker is presented in Table 2. In general, contaminant concentrations were low with the exception of DDT, endrin, dieldrin, mirex, and some heavy metals (arsenic, copper, and lead). In catfish, DDT, endrin and mirex were the predominant pesticides (Table 3). Regarding heavy metals in the edible flesh of catfish, arsenic, copper, and lead were predominant. As with the other species, arsenic and copper were the predominant heavy metals in shrimp (Table 4). Polychlorinated biphenyls (PCBs), expressed as either individual congeners or total PCBs, were low in all species examined.

Contaminant levels were compared to available U.S. Food and Drug Administration (FDA) action levels for pesticides, PCBs, and mercury. One percent of the catfish population and 9% of the shrimp sampled exceeded the FDA action level of 1.0 ppm for mercury in the edible portion of fish, shellfish, or crustaceans (Figure 1). Because of the paucity of information concerning U.S. standards for heavy metals other than mercury in fish, contaminant levels were compared to the mean of available international standards (Nauen 1983). Using international standards, 5 to 62% of the species exceeded arsenic standards, 1% of the croaker and catfish exceeded chromium, 2% of croaker exceeded selenium, 1 to 9% of croaker and catfish exceeded copper, and between 2 to 5% of croaker and catfish exceeded lead standards (Figure 2).

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Table 1. Contaminants analyzed for in edible fish and shellfish tissue

DDT and Metabolites

2 4′ DDD	4 4′ DDD
2 4′ DDE	4 4′ DDE
2 4′ DDT	4 4′ DDT

Chlorinated Pesticides

Aldrin	Heptachlor epoxide
Chlordane	Hexachlorobenzene
Dieldrin	Lindane
Endosulfan	Mirex
Endrin	Toxaphene
Heptachlor	Trans-nonachlor

Polychlorinated Biphenyl Congeners[a]

8	2 4′	126	3 3′4 4′5
18	2 2′5	128	2 2′3 3′4 4′
28	2 4 4′	138	2 2′3 4 4′5′
44	2 2′3 5′	153	2 2′4 4′5 5′
52	2 2′5 5′	170	2 2′3 3′4 4′5
66	2 3′4 4′	180	2 2′3 4 4′5 5′
77	3 3′4 4′	187	2 2′3 4′5 5′6
99	2 2′4 4′5	195	2 2′3 3′4 4′5 6
101	2 2′4 5 5′	206	2 2′3 3′4 4′5 5′6
105	2 3 3′4 4′	209	2 2′3 3′4 4′5 5′6 6′
118	2 3′4 4′5		

Heavy Metals

Aluminum	Mercury
Arsenic	Nickel
Cadmium	Selenium
Chromium	Silver
Copper	Tin
Lead	Zinc

[a]Nomenclature for polychlorinated biphenyl congeners follows that proposed by Ballschmitter and Zell (1980) and later adopted by the International Union of Pure and Applied Chemists (IUPAC).

Table 2. Overview of contaminant levels observed in edible flesh of Atlantic croaker

Contaminant	n	Mean	Observed Range
Pesticides (ng/g wwt)[a]			
DDD	84	0.38	0 - 49.4
DDE	84	0.58	0 - 8.9
DDT	84	0.62	0 - 6.5
Aldrin	84	0.30	0 - 2.8
Chlordane	84	0.18	0 - 2.4
Dieldrin	84	0.07	0 - 2.0
Endosulfan	84	0.06	0 - 2.3
Endrin	84	0.30	0 - 11.1
Heptachlor	84	0.00	0 - 0.0
Heptachlor epoxide	84	0.05	0 - 1.7
Hexachlorobenzene	84	0.08	0 - 6.1
Lindane	84	0.00	0 - 0.0
Mirex	84	0.15	0 - 10.5
Toxaphene	84	0.00	0 - 0.0
Trans-nonachlor	84	0.09	0 - 2.3
PCBs (ng/g wwt)[a]			
21 Congeners	59	0.32	0 - 91.3
Total PCBs	59	8.00	0 - 95.3
Heavy Metals (µg/g wwt)[b]			
Aluminum	89	7.23	0 - 36.1
Arsenic	89	0.59	0 - 7.0
Cadmium	89	0.01	0 - 0.2
Chromium	89	0.15	0 - 1.1
Copper	89	1.15	0 - 25.4
Lead	89	0.05	0 - 1.6
Mercury	89	0.11	0 - 0.7
Nickel	89	0.15	0 - 1.0
Selenium	89	0.44	0 - 1.4
Silver	89	0.01	0 - 0.1
Tin	89	0.07	0 - 1.1
Zinc	89	7.78	1 - 14.6

[a]Values expressed as ng/g wet weight.
[b]Values expressed as µg/g wet weight.

Table 3. Overview of contaminant levels observed in edible flesh of catfish

Contaminant	*n*	Mean	Observed Range
Pesticides (ng/g wwt)[a]			
DDD	89	0.20	0 - 18.5
DDE	89	2.13	0 - 104.8
DDT	89	1.11	0 - 20.7
Aldrin	89	0.59	0 - 22.5
Chlordane	89	0.20	0 - 4.2
Dieldrin	89	0.97	0 - 83.6
Endosulfan	89	0.00	0 - 0.0
Endrin	89	0.02	0 - 1.9
Heptachlor	89	0.06	0 - 2.6
Heptachlor epoxide	89	0.06	0 - 1.6
Hexachlorobenzene	89	0.07	0 - 6.3
Lindane	89	0.04	0 - 2.0
Mirex	89	0.06	0 - 2.4
Toxaphene	89	0.00	0 - 0.0
Trans-nonachlor	89	0.10	0 - 6.1
PCBs (ng/g wwt)[a]			
21 Congeners	81	0.31	0 - 20.6
Total PCBs	81	6.50	0 - 56.7
Heavy Metals (µg/g wwt)[b]			
Aluminum	101	10.9	0 - 88.6
Arsenic	101	5.69	0 - 39.9
Cadmium	101	0.00	0 - 0.0
Chromium	101	0.12	0 - 1.4
Copper	101	1.39	0 - 11.6
Lead	101	0.16	0 - 6.5
Mercury	101	0.19	0 - 1.6
Nickel	101	0.42	0 - 30.5
Selenium	101	0.19	0 - 0.6
Silver	101	0.01	0 - 0.1
Tin	101	0.01	0 - 0.1
Zinc	101	11.4	5 - 28.4

[a]Values expressed as ng/g wet weight.
[b]Values expressed as µg/g wet weight.

Table 4. Overview of contaminant levels observed in edible flesh of shrimp

Contaminant	*n*	Mean	Observed Range
Pesticides (ng/g wwt)[a]			
DDD	11	0.00	0 - 0.0
DDE	11	0.29	0 - 2.4
DDT	11	0.20	0 - 2.2
Aldrin	11	1.46	0 - 4.8
Chlordane	11	0.00	0 - 0.0
Dieldrin	11	0.00	0 - 0.0
Endosulfan	11	0.60	0 - 3.2
Endrin	11	0.00	0 - 0.0
Heptachlor	11	1.03	0 - 8.0
Heptachlor epoxide	11	0.23	0 - 2.5
Hexachlorobenzene	11	0.49	0 - 4.5
Lindane	11	0.00	0 - 0.0
Mirex	11	0.00	0 - 0.0
Toxaphene	11	0.00	0 - 0.0
Trans-nonachlor	11	0.14	0 - 1.5
PCBs (ng/g wwt)[a]			
21 Congeners	7	0.19	0 - 12.7
Total PCBs	7	6.94	0 - 14.6
Heavy Metals (µg/g wwt)[b]			
Aluminum	11	22.4	3 - 56.2
Arsenic	11	0.73	2 - 4.1
Cadmium	11	0.03	0 - 0.2
Chromium	11	0.17	0 - 0.3
Copper	11	8.58	6 - 18.3
Lead	11	0.03	0 - 0.1
Mercury	11	0.19	0 - 1.0
Nickel	11	0.11	0 - 0.2
Selenium	11	0.43	0 - 0.7
Silver	11	0.02	0 - 0.1
Tin	11	0.00	0 - 0.0
Zinc	11	15.6	14 - 18.7

[a]Values expressed as ng/g wet weight.
[b]Values expressed as µg/g wet weight.

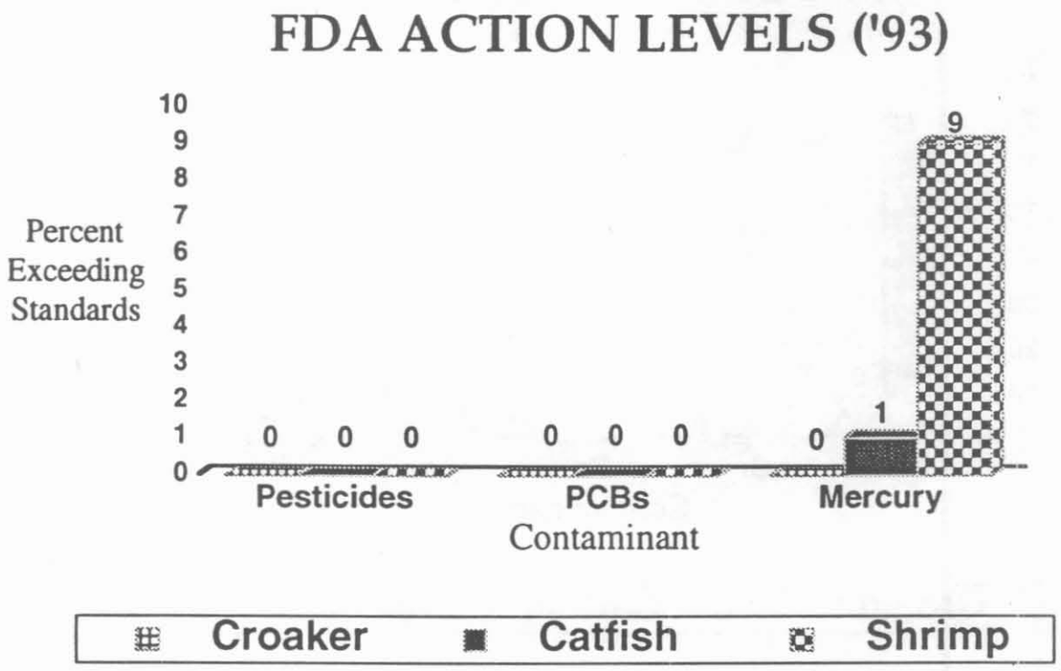

Figure 1. Comparision of tissue residue levels with FDA Action levels.

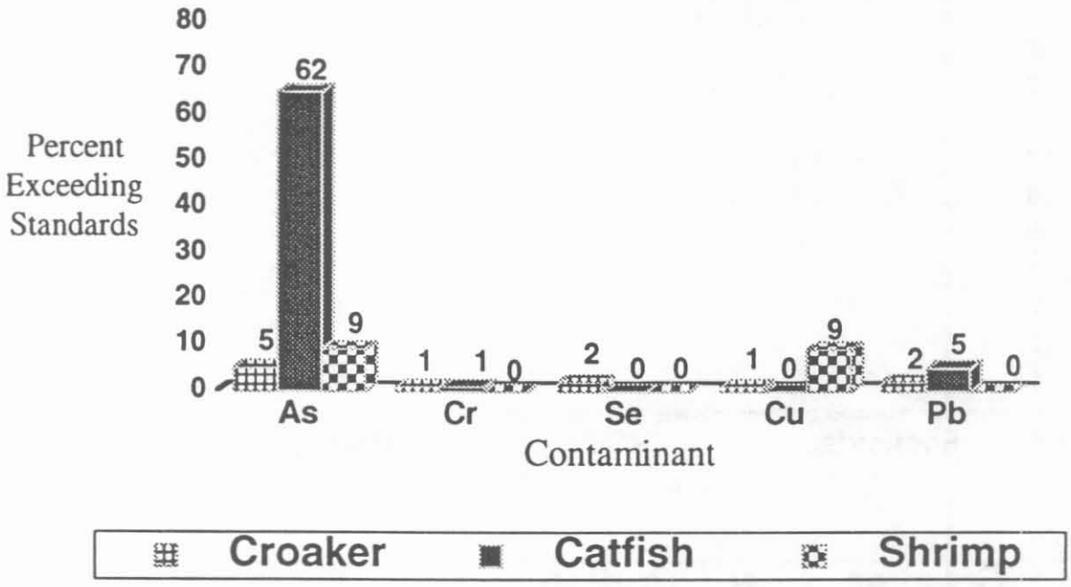

Figure 2. Comparison of tissue residue levels with International Standards (Nauen 1983).