EVALUATION OF TISSUE RESIDUES FOR THE ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM NEAR COASTAL - LOUISIANIAN DEMONSTRATION

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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, come 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.

This study represents the first year of data available for analytical chemistry evaluations which will encompass species (fish and shellfish) collected from the EMAP - Louisianian Province Demonstration Project for 1991.

MATERIALS AND METHODS

Sample Collection and Preparation

Sampling was conducted during the summer of 1991 (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 1991 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, 178 sites between Anclote Anchorge, 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 (*Arius felis*), 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 laboratory for subsequent analysis for the contaminants listed in Table 1.

Samples were received by the laboratory within 24 hours of collection. Upon receipt, samples were

inspected, logged-in to the laboratory sample tracking system, and stored (-20^{0}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 hours in a freeze dryer (Virtis). After 12 hours, the sample was again weighed and the percentage moisture was determined. Previous experiments had shown that 12 hours 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^{0}C for 24 hours) 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. In order to prepare samples for metals analysis (except mercury), 10 gm of tissue were placed in a 150 ml Fleaker (Pyrex) and 10 ml of concentrated nitric acid (J.T. Baker Instra-Analyzed) were added. A watch glass was placed on top of the Fleaker and the mixture was gently refluxed on a hot plate for 30 min. After refluxing, 1 ml of concentrated hydrochloric acid (J.T. Baker Instra-Analyzed) and 3 ml of concentrated sulfuric acid (J.T. Baker Instra-Analyzed) were added to the Fleaker and the volume was reduced to 2 to 3 ml by gentle heating. An additional 5 ml of concentrated nitric acid was added and the mixture was again refluxed for 30 min. If necessary an additional 3 ml of concentrated sulfuric acid were added. Following this addition, the volume was again reduced to 2 to 3 ml by gentle heating. An additional 5 ml of concentrated nitric acid were added and the mixture was refluxed for 30 min. Ten ml of 30% hydrogen peroxide (Fisher) were added in very small additions. The resulting solution was refluxed for 30 min. After this heating period, the digest was reduced to a volume of 5 ml by gentle heating. The digest was allowed to cool and then was volumetrically diluted to a final volume of 100 ml.

For mercury, 3 gm of tissue were placed in a 300 ml glass bottle (Wheaton), 5 ml of concentrated nitric acid were added, and the mixture was gently heated on a hot plate for 30 min. Following heating, the mixture was allowed to cool. Five ml of concentrated sulfuric acid, 15 ml of 5% potassium permanganate (Fisher), and 8 ml of potassium persulfate (Fisher) were added to the sample. The resulting mixture was allowed to remain at room temperature for at least 15 min. Following this time period, 6 ml of a 12% hydroxylamine hydrochloride (Fisher) -12% sodium chloride (Fisher) solution were added to the sample. Finally, 61 ml of DI water were added to the sample and the sample was gently agitated to ensure thorough mixing of all reagents and the sample.

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^{0}C and the detector temperature was 310^{0}C. Temperature programming was used to chromatograph the samples: an initial oven temperature of 150^{0}C was held for 40 min, raised to 195^{0}C at 1^{0}C/min, held at 195^{0}C for 20 min, raised to 220^{0}C at 1^{0}C/min, held at 220^{0}C for 2 min, raised to 280^{0}C at 3^{0}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 DDD, DDT, mirex, toxaphene and some heavy metals (aluminum, arsenic, copper, and zinc). In catfish, DDD, DDT, mirex and toxaphene were the predominant pesticides (Table 3). Regarding heavy metals in the edible flesh of catfish, aluminum, arsenic and zinc were present in the highest concentrations. Although DDT and mirex were present in shrimp, no toxaphene residues were detected (Table 4). Aluminum, arsenic, chromium, and zinc were the predominant heavy metals in shrimp. 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 (Fig. 1). Only 1% of the catfish population sampled exceeded the FDA action level of 1.0 ppm for total mercury in the edible portion of fish, shellfish, or crustaceans. 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, 3 to 8% of Atlantic croaker, catfish, as well as shrimp exceeded arsenic standards (Fig. 2). Additionally, 4% of shrimp exceeded chromium standards and 2% of catfish exceeded zinc standards.

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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	53	3.21	0 - 16.0
DDE	53	0.62	0 - 3.5
DDT	53	4.00	0 - 24.2
Aldrin	53	0.18	0 - 3.2
Chlordane	53	0.75	0 - 8.2
Dieldrin	53	1.06	0 - 26.2
Endosulfan	53	0.08	0 - 1.7
Endrin	53	0.68	0 - 22.5
Heptachlor	53	0.29	0 - 5.7
Heptachlor epoxide	53	0.73	0 - 16.7
Hexachlorobenzene	54	1.64	0 - 77.4
Lindane	54	0.00	0 - 0.0
Mirex	53	5.14	0 - 88.5
Toxaphene	58	167.07	0 - 1800
Trans-nonachlor	53	0.28	0 - 1.3
PCBs (ng/g wwt)[a]			
21 Congeners	53 - 58	0.58	0 - 40.6
Total PCBs		2.85	0 - 62.5
Heavy Metals (µg/g wwt)[b]			
Aluminum	38	1.74	0 - 6.9
Arsenic	38	0.33	0 - 2.1
Cadmium	38	0.01	0 - 0.1
Chromium	38	0.04	0 - 0.3
Copper	38	0.41	0 - 5.3
Lead	38	0.03	0 - 0.3
Mercury	38	0.03	0 - 0.4
Nickel	38	0.09	0 - 0.3
Selenium	38	0.13	0 - 0.3
Silver	38	0.10	0 - 1.8
Tin	38	0.16	0 - 0.7
Zinc	38	6.43	1 - 11.8

[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	104	11.64	0 - 207.4
DDE	104	1.15	0 - 12.2
DDT	104	7.47	0 - 39.4
Aldrin	104	0.29	0 - 2.7
Chlordane	104	0.92	0 - 6.1
Dieldrin	104	1.05	0 - 24.2
Endosulfan	105	0.10	0 - 1.8
Endrin	104	0.51	0 - 10.1
Heptachlor	104	0.22	0 - 5.7
Heptachlor epoxide	104	0.62	0 - 5.7
Hexachlorobenzene	107	0.32	0 - 4.0
Lindane	105	0.02	0 - 4.1
Mirex	104	3.17	0 - 30.7
Toxaphene	111	36.15	0 - 1400
Trans-nonachlor	104	0.88	0 - 4.3
PCBs (ng/g wwt)[a]			
21 Congeners	104 - 110	0.83	0 - 19.5
Total PCBs		10.56	0 - 67.9
Heavy Metals (µg/g wwt)[b]			
Aluminum	80	6.35	0 - 105.1
Arsenic	80	1.03	0 - 10.1
Cadmium	80	0.02	0 - 0.4
Chromium	80	0.05	0 - 0.8
Copper	80	0.89	0 - 10.3
Lead	80	0.01	0 - 0.4
Mercury	86	0.06	0 - 1.2
Nickel	80	0.08	0 - 0.7
Selenium	80	0.06	0 - 0.4
Silver	80	0.04	0 - 0.3
Tin	80	0.21	0 - 1.2
Zinc	80	13.45	1 - 234.0

[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	37	0.78	0 - 4.9
DDE	37	0.22	0 - 1.7
DDT	37	6.95	0 - 74.0
Aldrin	37	0.14	0 - 1.6
Chlordane	37	0.17	0 - 1.9
Dieldrin	37	0.16	0 - 1.6
Endosulfan	37	0.00	0 - 0.0
Endrin	37	0.51	0 - 12.8
Heptachlor	37	0.00	0 - 0.0
Heptachlor epoxide	37	0.50	0 - 3.9
Hexachlorobenzene	37	0.37	0 - 2.5
Lindane	37	0.00	0 - 0.0
Mirex	37	3.84	0 - 43.5
Toxaphene	37	0.00	0 - 0.0
Trans-nonachlor	37	0.18	0 - 1.3
PCBs (ng/g wwt)[a]			
21 Congeners	37	0.26	0 - 16.1
Total PCBs		0.60	0 - 30.3
Heavy Metals (µg/g wwt)[b]			
Aluminum	24	18.76	0 - 78.5
Arsenic	24	0.74	0 - 3.9
Cadmium	24	0.03	0 - 0.3
Chromium	24	0.32	0 - 6.1
Copper	24	3.09	0 - 9.6
Lead	24	0.01	0 - 0.3
Mercury	24	0.05	0 - 0.3
Nickel	24	0.50	0 - 9.0
Selenium	24	0.07	0 - 0.3
Silver	24	0.04	0 - 0.3
Tin	24	0.31	0 - 1.1
Zinc	24	10.56	1 - 18.8

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

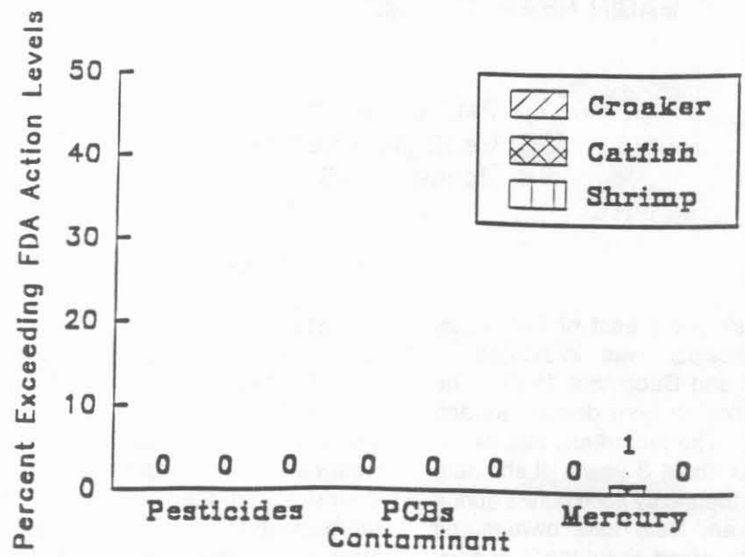

Fig. 1. Percent of target species exceeding FDA action levels for pesticides, polychlorinated biphenyls (PCBs) and mercury.

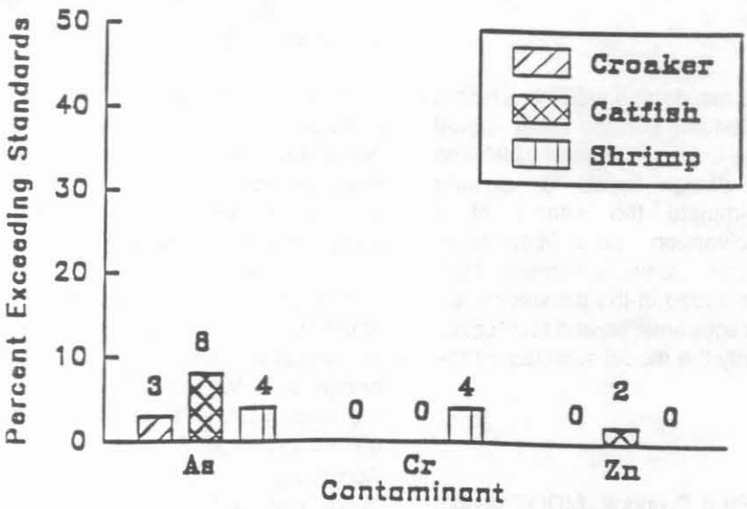

Fig. 2. Percent of target species exceeding international standards for arsenic (As), chromium (Cr), and zinc, (Zn).