

ASSESSMENT OF CONTAMINANT LEVELS IN FISH AND SHELLFISH FROM AND THE GULF OF MEXICO

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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 - Near Coastal 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.

While it is difficult to prioritize the problems and environmental concerns present in the Gulf, existing and potential problems do exist. The Toxics and Pesticides Subcommittee of the U.S. Environmental Protection Agency's Gulf of Mexico Program has identified the following areas to be addressed:

- Determine impact of pesticides and other toxic substances on nontarget organisms, and coastal and marine systems.
- Monitor occurrence and effects of toxic substances and pesticides on organisms and coastal and marine ecosystems.
- Improve risk assessment techniques for determining impacts of toxic substances and pesticides on marine organisms and ecosystems.

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 - Near Coastal Project for 1992.

MATERIALS AND METHODS

Sample Collection and Preparation

Sampling was conducted during the summer of 1992 (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 1992 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 (*Arius felis*), gafftopsail catfish (*Bagre marinus*), blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), 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. Photon 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, mirex, and some heavy metals (aluminum, arsenic, cadmium, and zinc). In catfish, DDT and mirex were the predominant pesticides (Table 3). Regarding heavy metals in the edible flesh of catfish, aluminum, arsenic, mercury, selenium, tin, and zinc were predominant. As with the other species, DDT and mirex were detected in shrimp (Table 4). Aluminum, arsenic, copper, selenium, 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. Only 1% of the catfish population sampled exceeded the FDA action level of 1.0 ppm for 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, 15% of catfish exceeded cadmium standards and 1 to 4% of Atlantic croaker and catfish exceeded cadmium standards and 2 to 4% of catfish and shrimp exceeded selenium 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

^aNomenclature 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
<u>Pesticides (ng/g wwt)^a</u>			
DDD	97	0.20	0 - 6.1
DDE	97	0.73	0 - 9.3
DDT	97	1.75	0 - 36.6
Aldrin	97	0.14	0 - 8.6
Chlordane	97	0.28	0 - 3.5
Dieldrin	97	0.05	0 - 3.5
Endosulfan	97	0.05	0 - 2.0
Endrin	97	0.05	0 - 1.1
Heptachlor	97	0.08	0 - 3.0
Heptachlor epoxide	97	0.10	0 - 2.9
Hexachlorobenzene	97	0.04	0 - 2.9
Lindane	97	0.01	0 - 1.3
Mirex	97	3.15	0 - 42.1
Toxaphene	97	0.00	0 - 0.0
Trans-nonachlor	97	0.15	0 - 5.6
<u>PCBs (ng/g wwt)^a</u>			
21 Congeners	97	0.38	0 - 30.3
Total PCBs	76	9.24	0 - 98.9
<u>Heavy Metals (µg/g wwt)^b</u>			
Aluminum	79	2.13	0 - 4.1
Arsenic	80	0.22	0 - 0.9
Cadmium	73	0.16	0 - 0.7
Chromium	78	0.16	0 - 0.5
Copper	80	0.37	0 - 1.3
Lead	73	0.08	0 - 0.2
Mercury	82	0.07	0 - 0.4
Nickel	79	0.24	0 - 0.8
Selenium	80	0.15	0 - 0.6
Silver	75	0.13	0 - 0.5
Tin	80	0.31	0 - 0.9
Zinc	80	3.05	1 - 5.8

^aValues expressed as ng/g wet weight.

^bValues expressed as µg/g wet weight.

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

Contaminant	n	Mean	Observed Range
<u>Pesticides (ng/g wwt)^a</u>			
DDD	154	0.30	0 - 13.0
DDE	157	1.81	0 - 20.9
DDT	157	2.58	0 - 37.8
Aldrin	157	0.18	0 - 16.7
Chlordane	157	0.29	0 - 79.9
Dieldrin	157	0.16	0 - 5.0
Endosulfan	157	0.03	0 - 2.8
Endrin	157	0.27	0 - 12.5
Heptachlor	157	0.46	0 - 49.2
Heptachlor epoxide	157	0.08	0 - 2.3
Hexachlorobenzene	157	0.04	0 - 2.5
Lindane	157	0.10	0 - 15.4
Mirex	157	3.15	0 - 72.6
Toxaphene	157	0.00	0 - 0.0
Trans-nonachlor	157	0.16	0 - 3.2
<u>PCBs (ng/g wwt)^a</u>			
21 Congeners	157	0.51	0 - 44.8
Total PCBs	137	10.46	0 - 79.9
<u>Heavy Metals (µg/g wwt)^b</u>			
Aluminum	137	2.01	0 - 16.7
Arsenic	135	1.05	0 - 10.3
Cadmium	127	0.08	0 - 0.5
Chromium	136	0.01	0 - 0.7
Copper	136	0.36	0 - 2.0
Lead	127	0.06	0 - 0.3
Mercury	135	0.13	0 - 1.2
Nickel	136	0.24	0 - 2.2
Selenium	136	0.24	0 - 1.3
Silver	129	0.09	0 - 0.5
Tin	136	0.42	0 - 2.6
Zinc	136	4.21	1 - 18.2

^aValues expressed as ng/g wet weight.

^bValues expressed as µg/g wet weight.

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

Contaminant	n	Mean	Observed Range
<u>Pesticides (ng/g wwt)^a</u>			
DDD	61	0.05	0 - 4.5
DDE	61	0.01	0 - 0.5
DDT	61	1.73	0 - 14.0
Aldrin	61	0.01	0 - 0.7
Chlordane	61	0.00	0 - 0.0
Dieldrin	61	0.00	0 - 0.0
Endosulfan	61	0.00	0 - 0.0
Endrin	61	0.00	0 - 0.0
Heptachlor	61	0.14	0 - 4.2
Heptachlor epoxide	61	0.01	0 - 0.4
Hexachlorobenzene	61	0.00	0 - 0.0
Lindane	61	0.00	0 - 0.0
Mirex	61	1.47	0 - 16.0
Toxaphene	61	0.00	0 - 0.0
Trans-nonachlor	61	0.00	0 - 0.0
<u>PCBs (ng/g wwt)^a</u>			
21 Congeners	61	0.09	0 - 16.0
Total PCBs	50	2.28	0 - 27.4
<u>Heavy Metals (µg/g wwt)^b</u>			
Aluminum	52	3.00	0 - 5.1
Arsenic	53	0.23	0 - 1.4
Cadmium	52	0.06	0 - 0.4
Chromium	53	0.10	0 - 0.4
Copper	53	1.06	0 - 2.4
Lead	52	0.04	0 - 0.2
Mercury	65	0.02	0 - 0.2
Nickel	53	0.16	0 - 0.4
Selenium	53	0.25	0 - 1.1
Silver	52	0.14	0 - 0.5
Tin	53	0.38	0 - 1.0
Zinc	53	4.04	1 - 11.5

^aValues expressed as ng/g wet weight.

^bValues expressed as µg/g wet weight.