Exposure Investigation

Isla de Vieques
Edible Fish

Vieques, Puerto Rico

May 14, 2002

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry
Division of Health Assessment and Consultation
Atlanta, Georgia 30333

Table of Contents

Objective

The purpose of this exposure investigation (EI) is to determine if the fish in Vieques are contaminated with metals and explosive compounds.

This EI was initiated because (1) U.S. Navy activities on Vieques can release explosive compounds and metals (natural or man-made) into the environment, (2) fish are known to accumulate metals from the environment, (3) people are sometimes exposed to metals when they eat fish, and (4) Vieques residents and healthcare providers are concerned about metal exposures.

This exposure investigation was conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) together with assistance from the Environmental Protection Agency=s Environmental Response Team (EPA/ERT).

Background

The Island of Vieques is located approximately 6 miles off the southeast end of the island of Puerto Rico, in the Caribbean Sea. Vieques is approximately 21 miles long and 5 miles wide at its widest point, with an approximate area of 55 square miles. The east end of the island (approximately 50% of the total land area) is occupied by the U.S. Navy, and is used for military training. The western portion of the island was formerly occupied by the Navy, but became a national park in early 2001.

Vieques has been used by the Navy for military training for 60 years. Training has included live impact bombing (aerial and ship-to-shore) on the tip of the eastern end of the island. Residents of Vieques have expressed concern that the residues from the exploding ordnance have contaminated the soil, sediment, water, fish, and invertebrates. Chemicals of interest (COIs) include heavy metals (particularly arsenic, mercury, lead, cadmium and zinc), and explosive compounds (including octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine [HMX], and hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX]).

***Demographics*:**

The central ⅓ of the island is occupied by civilians. Approximately 9,400 civilians reside mainly in two towns, Isabel Segunda on the north shore, and Esperanza on the south shore (Figure 1).

Justification for EI

A resident petitioned ATSDR to evaluate exposures to potential contaminants produced by the Navy activities on Vieques. Also, existing data are insufficient to evaluate such exposures.

The major components of Navy’s bombing activities are metals and explosives. Residents and health care providers are concerned about metals measured in the hair and blood of some Vieques residents. Metal exposure commonly occurs by eating metal-contaminated foods. While many people eat local fish, crabs, and conch, very little metal data is available for these species.

Some citizens were concerned about mercury and arsenic exposures. It has been suggested that older bombs contained small amounts of mercury and arsenic and that the rocks on part of the island contained arsenic. Some theorized that the arsenic in the rocks could be broken down by bombs, that both mercury and arsenic could leach into the waters nearby, and that the fish in these waterways cold have become contaminated.

In this EI, accepted methods were used to determine metal concentrations in local seafood. And if warranted, similarly prudent measures were recommended to reduce metal exposures. Seafood samples were also analyzed for residues of chemicals known to be present in explosive ordnance used by the Navy. For comparative purposes, samples were collected from six locations, including a fish market and a reference area.

Methods

The field work for this EI was conducted from July 15-21, 2001. Samples were collected and prepared in Vieques. Fish and invertebrates were collected from six locations: four near shore areas to the north and south of Vieques, one reference area on the west end of Vieques, and a fish market in the town of Isabel Segunda (**Figure 1**).

Sampling Locations

Location 1: Two small, near-shore reefs (North LIA 1, and North LIA 2) to the north of the Navy Live Impact Area (LIA) on the east end of Vieques.

Location 2: Two sunken Navy vessels, used for military target practice. A barge and a ship, approximately 100 meters apart, near shore to the south of the Navy LIA on the east end of Vieques.

Location 3: Three near-shore reefs to the south of the town of Esperanza (on the south shore of Vieques). Fish were collected at Bucky Reef and Patti Reef, and conch were collected from the seagrass bed north of Arena Reef.

Location 4: Two near-shore reefs (Isabel 1 and Isabel 2) to the northwest of the town of Isabel Segunda (on the north shore of Vieques), and the Mosquito Pier to the west of Isabel Segunda.

Location 5: The Johan Pescaderia (fish market) in the town of Isabel Segunda.

Location 6: The reference location: An unnamed near-shore reef (West End) off of the southwest end of Vieques, in the vicinity of the Monte Pirata Conservation Zone. Conch were collected from a seagrass bed 500 meters northeast of the reef.

Field personnel collected the fish and invertebrate samples by spear, trap, hand, hook and line, and market purchase. The latitude/longitude of the approximate center of each sampling location was determined using a global positioning system (GPS) unit.

Quality Assurance

At each location, attempts were made to collect five of each of the following eight types of fish: yellowtail snapper (*Ocyurus chrysurus*), mutton or lane snapper (*Lutjanus* sp.), grouper/red hind/rock hind/coney (*Epinephelus* sp.), grunt (*Haemulon* sp.), parrotfish (Scaridae family), porgy (Sparidae family), goatfish (Mullidae family), or bonito (Scombridae family). These fish are listed in the order most commonly consumed by residents of Vieques (UMET 2000). Attempts were made to catch fish of the same five species at each sampling location. At each location, attempts were also made to collect five queen conch (*Strombus gigas*), and spiny lobster (*Panulirus argus*). On shore, adjacent to each sampling location, the plan was to collect a sufficient number of both the blue land crab (*Cardisoma guanhumi*) and the fiddler crab (*Uca* sp.) to meet the tissue mass requirements for five replicates of the desired chemical analyses.

Because fish slowly accumulate metals during their life as they grow, efforts were made to collect organisms of the same approximate size (by species) at each location so that final results could more easily be compared.

The specimens were processed according to Standard Operating Procedures for Fish Handling and Processing (USEPA 1990a). Each fish or shellfish was weighed, measured, and examined both externally and internally for obvious abnormalities. Tissue was cut from the specimen, wrapped in plastic wrap, placed in a sealable plastic bag, and placed on wet ice until it could be frozen. The tissues were shipped on dry ice, via overnight delivery to Campuchem Laboratories at Cary, NC for analysis of the target analyte list (TAL) metals,[1] explosive compounds,[2] percent lipids, and percent moisture. Further discussion is contained in Appendix A.

Statistical Analysis

[1]Analyzed with atomic absorption (EPA method 6010B, SW-846).

[2]Analyzed with high pressure liquid chromatography (EPA method 8330, SW-846).

Species of similar size were selected during the sampling phase to compare the data from one site to another. Statistical analysis[3] was performed on each species to find if there were any locations where the concentrations were higher. When one can determine that one location has higher measurements than others, it sometimes suggests a source of contamination is nearby. The analysis determines the degree to which the data from each species in each location can be compared–this is called testing the normality and homogeneity of variance. Then the grouped data from one location can be compared to that elsewhere. This is known as a multi-variate analysis of the variance. The description of each statistical test and the results could be found in USEPA 2001.

The metals most common in bombs (iron, aluminum, zinc, etc.) were chosen to be tested, as well as the trace metals that posed a concern to the community (arsenic and mercury). Should both the common metals (which are not harmful) and the trace metals (which could be harmful), be significantly higher near the bombing exercises, this would be evidence of contamination from bombing. If in any single location a metal is both significantly higher and elevated (to levels above 3 times the average)[4], then it is likely that there is a localized source of contamination of that metal.

Results

At all sampling locations, fish were plentiful and appeared to be in good health, but were generally small. Though yellowtail snapper was listed as the most commonly caught and consumed fish in Vieques (UMET 2000), very few Yellowtail were seen at any of the sampling locations, and those that were approachable were quite small (<30 cm). It appears that the reefs around Vieques are heavily fished. For this reason, many of the fish collected by field personnel were small, and it was not possible to collect five individuals of each of five species at every sampling location, as originally planned.

<u>Explosive Compounds</u>

---

[3]Probabilistic statistical analysis, evaluated at $\alpha = 0.05$. Tested for normality and homogeneity of variance, then, multi-variate analysis of variance, and multiple comparison of the means.

[4]Factors of 3 to 5 are commonly used to indicate unusually elevated average levels as environmental data naturally varies.

Trace levels of explosive residues were found. No explosive compounds were detected in fish, conch, lobster, or land crab tissues from any of the field-sample locations. Still, HMX and RDX were shown to be present in the fiddler crab samples, and RDX was shown to be present in the trunkfish sample from the fish market. But because they were below the (1 mg/kg) method detection limit, these concentrations were listed as estimates.

TAL Metals

Fish and invertebrate tissues were analyzed for TAL metals. TAL analysis results refer to dry tissue. Analytical results are summarized in **Table 1** (in dry weight). The results were adjusted to wet-weight to evaluate the health significance.

Compared to fish collected elsewhere in the U.S., no metals are found at unusually high levels. Arsenic is commonly found in seafood and varies widely among species. Levels of total arsenic were higher in the lobster and goatfish and much lower in the other species. The data was wet-weight adjusted using the percent of tissue moisture. The average wet-weight arsenic levels are given with the appropriate comparison values given in **Table 2**.

Table 2: Wet Weight Arsenic Concentrations in Tissue (in mg/kg)

Species	Concentration		Comparison level[5]
	Mean	(Range)	
Hind	2.3	(0.13 - 8.7)	4.5
Grunt	2.8	(0.90 - 10.1)	4.5
Goatfish	8.3	(5.2 - 11.5)	4.5
Parrotfish	2.6	(0.13 - 13.1)	4.5
Snapper	2.5	(0.13 - 21.4)	4.5
Trunkfish	29 [6]	(no range)	NA
Conch	4.0	(1.2 - 7.0)	86 [7]
Lobster	33	(23.4 - 48.3)	76 [8]
Land crab	0.35	(0.13 - 2.5)	76 [5]
Fiddler crab	2.8	(2.3 - 3.2)	76 [5]

[5]Average arsenic values in US fin fish (Bennet 1986; Schroeder and Balsa 1966)

[6]Only one trunkfish was available for analysis.

[7]FDA Guidance for molluscan bivalves (conch are mulluscs, but not bivalves)

[8]FDA Guidance for crustacea

NA	=	No appropriate comparison value could be obtained for this species. No normal average measurements were found in literature, and the species is unlike most fin fish in that it has a hard, shell- like exterior.

The highest wet-weight arsenic concentration was 48 mg/kg found in lobster (the average lobster was 33 mg/kg). The highest level found in snapper, a frequently eaten fish, was 21 mg/kg (average was 2.5 mg/kg). Arsenic levels were lowest in the land crabs, 0.35 mg/kg.

Total mercury, also found commonly in fish, was highest in the grunt, with a mean concentration of 0.07 mg/kg and a maximum of 0.33 mg/kg (wet-weight).

Methyl Mercury

One tissue sample from each of four target species collected from the fish market (Location 5) was submitted for methyl mercury analysis. Methyl mercury ranged from 0.018 - 0.0823 mg/kg (wet-weight). This is 42-78% of the total mercury in the fish, less than the 85% estimate proposed by (Jones and Slotten 1996). Analytical results can be found in the attached report and summarized with the appropriate comparison values below (in mg/mg).

Table 3: Wet Weight Methyl Mercury Concentrations in Tissue (in mg/kg)

Species	Concentration	FDA Action Level
Grouper	0.0625	1.0
Grunt	0.0823	1.0
Snapper	0.018	1.0
Lobster	0.0194	1.0

Discussion

During the investigation, a sufficient number of fish from the different locations were collected to determine that no appreciable amounts of metals or explosives were entering the seafood supply. In this section measurements on the fish collected in Vieques will be compared with fish collected elsewhere in the U.S. What these data might mean to someone eating fish in Vieques will then be discussed. Finally, statistics will be used to determine any locations where chemicals might be at higher levels on the island; this is done as an indicator of whether bombing activities are contaminating the fish.

It is important to note that the chemicals are reported by the laboratory as dry weight concentrations (except for methyl mercury), which means that water was removed from the sample before analysis. Using the percent moisture determinations, the dry weight laboratory concentrations were adjusted to wet-weight concentrations to evaluate the health implications. This allows for direct comparisons with metal residues in fish/seafood reported in other studies or investigations and for direct comparisons with other dietary information.

Heath implications of explosive compounds

Of the several explosive compounds analyzed, traces of RDX were found in the trunkfish and HMX and RDX in some of the fiddler crabs. But whether the fiddler crabs had the HMX and RDX on the shell, in the shell, or in the meat could not be determined.

None of the HMX and RDX measurements are hazardous (discussed below). People seldom eat either trunkfish or fiddler crabs, and cooking will reduce the levels of RDX and HMX. Also, the collected specimens were analyzed uncooked and neither of the species is eaten uncooked. The trunkfish needs to be cooked well because it is naturally poisonous and is quite hard. Similarly, no meat can be taken from a 2-inch fiddler crab without cooking. Furthermore, the fiddler crabs caught were from the LIA which is not accessible to the general public.

HMX exposure implication
ATSDR has estimated an exposure level believed to be safe for HMX (ATSDR 1997). This safe level is equal to a dose of 1 mg/day to a 20 kg child for several days.[9] Because HMX was never above 1 mg/kg (dry weight) in any of the samples, a 20 kg child is safe eating 1 kg of fiddler crabs a day (of any of the samples).

RDX exposure implication
ATSDR has also estimated an exposure level believed to be safe for RDX (ATSDR 1995). This safe level is equal to a dose of 0.6 mg/day to a 20 kg child for several days.[8] Because RDX was never above 0.6 mg/kg (dry weight) in any of the samples, a 20 kg child is safe eating 1 kg of trunkfish or fiddler crab a day (of any of the samples).

Heath implications of metals

The metal concentrations[10] were lower than those found in fish caught in the tidal waters off of the continental U.S. and the levels found do not pose a public health hazard.

Fish are, generally, a major source of people=s exposure to metals because fish accumulate the metals present in their environment (ATSDR 1999; ATSDR 2000). Many metals are essential nutrients, most are naturally occurring with low toxicity, and some—in the case of gross contamination—are hazardous. Many metals are found in different forms in the environment, but only certain forms of metals are toxic. For example, methyl mercury is the more toxic form of mercury and inorganic arsenic is the more toxic form of arsenic. In fish, most of the mercury is the

[9]Applicable to continuous exposures up to 364 days.

[10]Mercury, arsenic, lead, manganese, beryllium, iron, and other metals provided in Table 1.

more toxic methyl mercury and most of the arsenic is the non-toxic organic arsenic.

The levels of total arsenic measured in the fish were about the same as those found in the U.S. coastal waters. Arsenic averaged 4 mg/kg in the fish collected in Vieques and averaged between 4 and 5 mg/kg in the fish collected elsewhere in the U.S.(Bennett 1986; Schroeder and Balsa 1966).

Arsenic guidance levels have only been set for shellfish because they are known to accumulate arsenic. The FDA has set a 76 mg/kg guidance for lobster and crabs (crustacea) and 86 mg/kg for molluscs (molluscan bivalves). The highest lobster concentration collected on Vieques was 48 mg/kg and only a few individuals within the other species had levels near those of the lobster. The trunkfish had 29 mg/kg arsenic and the highest snapper had 21 mg/kg arsenic. These individual levels are high (compared to the average), but it is not unusual to have some fish with these levels (NAS 1977). The remaining fish had much lower levels, with an average about the same as elsewhere in the U.S. People who eat the average fish from Vieques will get about as much total arsenic as those who eat canned tuna or fish sticks (Tao and Bolger 1999). Those who eat more lobster than average could have higher arsenic exposure, but this exposure is not expected to pose a health hazard.

Generally, inorganic arsenic, the toxic form of arsenic, accounts for 1.5% of the total arsenic in fish and 20% of arsenic in shellfish, but this percentage varies widely (ATSDR 2000). By using the heath protective value of 20% inorganic arsenic, one can conclude that none of the Vieques seafood collected had hazardous levels of arsenic.

Lobster example: 33 mg/kg x 20% = 6.6 mg/kg

Nevertheless, because the actual percentage of inorganic arsenic is unknown, only a few specific recommendations to reduce inorganic arsenic exposures can be made. The parrotfish, hind, snapper, and grunt all averaged below 3 mg/kg (total arsenic). Thus they should have low inorganic arsenic levels (as well). And even though shell fish tend to accumulate inorganic arsenic more than fin fish, the crab are expected to be low because the total arsenic values were low
(0.35 mg/kg).

The levels of total mercury measured in the Vieques seafood were lower than levels found in the U.S. coastal waters (ATSDR 1999). In Vieques, methyl mercury was measured at concentrations from 0.018 to 0.0823 mg/kg with a maximum total mercury level of 0.33 mg/kg and a mean of 0.07 mg/kg. Mean total mercury levels found in the northeastern U.S. are 0.45 mg/kg and in Florida are 0.64 mg/kg.[11] The FDA has set a wet basis action level of 1 mg/kg for total mercury—assuming that most mercury is methyl mercury. Fish in many states exceed this level (ATSDR 1999), but none caught in Vieques exceeded it. Of the fish collected in Vieques, the maximum level of total mercury (0.33 mg/kg) was found in the grunts—lower than (the 1 mg/kg) action level. Also, the rest of the species were much lower (averaging 0.07 mg/mg). Although, the

[11]It should be noted, however, that species can vary substantially.

mercury levels in the grunts do not pose a public health hazard, those wishing to reduce their mercury exposure may choose to eat another species. Parrotfish, snapper, lobster, land crabs, and conch had lower mercury levels than did grunts.

Statistical Analysis of COI Tissue Concentrations

Although no metals were unusually high in the sample fish when compared to levels elsewhere, statistical tests were used to determine how the metals in fish compared from one location of the island to another. If one location has higher measurements, it sometimes suggests a source of contamination is nearby. The analysis determines the degree to which the data from each species in each location can be compared; this is known as testing the normality and homogeneity of variance. Then the grouped data from one location to data elsewhere can be compared; this is known as a multi-variate analysis of the variance. The description of each statistical test and the results could be found in USEPA 2001. The statistical data has been presented here by coloring maps to show where one group of fish had higher concentrations than elsewhere. Because only data that passed the first test could be used, the decision was made to show those sites to which the higher levels compared. Thus if grouper had higher levels of iron to the south than, for example, to the west, and to the fish market, a green symbol was placed to the south, and a black symbol to the west and at the fish market, but nothing was placed at the locations where comparisons could not be made. A detailed interpretation is provided in Appendix A and is summarized below.

> There are no signs of gross contamination. Mercury and lead were not significantly higher in any location. Some metals were significantly higher in a few locations. Arsenic was more related to fish type than location (not unique to Vieques).
>
> **Figure 2** is a map showing the locations where any species contained metals other than arsenic; they are shown as significantly high in green or low in black.[12] All species[13] were included in this figure, but arsenic data from all the species were removed because it did not follow the same pattern as many of the other metals. Although the figure reveals that the levels on the west side are significantly lower than elsewhere, the values are not much lower (See Table 1). Metals, most notably aluminum and iron, are significantly higher in more species caught to the south of the island (south of the LIA and Esperanza), but not much higher than elsewhere. There is no clear indication that a contaminant source is present despite the higher average levels in the south. But the significantly lower average level to the west is worthy of mention.

[12]The figure is biased toward the more abundant species and gives equal weight to any measurement that was statistically significant no matter how much higher or lower they were.

[13]All species that were caught in sufficient numbers in at least 2 locations to facilitate statistical analysis (including: grouper, grunt, conch, land crab, snapper, parrotfish).

Figure 3 is the same map, but marked with the location and species that had significant arsenic values, i.e., high in green low in black. Because no location appears to have significantly high or low arsenic levels, there is no apparent source of arsenic.

Three general statements can be made after reviewing the two maps: (1) there is no sign of localized arsenic, mercury, or lead contamination in the fish, (2) the fish in the least-disturbed region (to the west) have the lowest metal levels, and (3) these metals are higher in the fish caught to the south of the island.

Assumptions and uncertainty are discussed in Appendix C.

Conclusions

1. There is no apparent health hazard posed by metals or explosives from eating seafood caught near Vieques.

   Metal concentrations were about the same or lower than those found in tidal waters of the continental U.S. and no metals were found above the FDA=s recommended levels. Explosive residues were all below the method detection limits.

2. There are no signs of mercury or arsenic contamination. Arsenic and mercury were low on average, but higher in certain species.

   Arsenic was higher in the lobster (not unique to Vieques).
   Mercury was higher in the grunts (not unique to Vieques).

Recommendations

None.

References

Agency for Toxic Substances and Disease Registry. 1995. Toxicological profile for RDX. Atlanta: US Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 1997. Toxicological profile for HMX. Atlanta: US Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 1999. Toxicological profile for mercury. Atlanta: US Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 2000. Toxicological profile for arsenic. Atlanta: US Department of Health and Human Services.

Colin, PL. 1978. Caribbean reef invertebrates and plants, Neptune City, NJ: T.F.H. Publications, Inc. Ltd.

Bennett BG. 1986. Exposure assessment for metals involved with carcinogenesis. IARC. Sci Publ 71(8):115-127.

Humann P. 1994, Reef fish identification; Florida, Caribbean, Bahamas. Jacksonville, FL: New World Publications, Inc.,

Jones AB and Slotten D.G. 1996. Mercury effects, sources, and control measures. A special study of the San Francisco Estuary Regional Monitoring Program. Richmond, CA: San Francisco Estuary Institute.

NAS. 1977. Arsenic—drinking water and health. Washington DC: National Academy of Sciences. p. 316-344, 428-30.

Schroeder HA and Balsa JJ. 1966. Abnormal trace metals in man. Arsenic J. Chron. Dis. 19:85-106.

Tao SS-H and Bolger PM. 1999. Dietary intakes of arsenic in the United States. Food Audit Contam 16:465-72.

[UMET] Universidad Metropolitana. 2000. Toxicological survey of heavy metals in fish populations, Vieques Island. Universidad Metropolitana, School of Environmental Matters.

US Environmental Protection Agency. 1990. Quality assurance/quality control guidance for removal activities. Sampling QA/QC Plan and data validation procedures. Washington, DC: EPA/540/G-90/004.

US Environmental Protection Agency. 1990a. Fish handling and processing. Standard operating procedure (SOP) # 2039 response engineering and analytical contract (REAC). Edison, NJ: US Environmental Protection Agency, Office of Emergency and Remedial Response.

US Environmental Protection Agency. 2001. Field data summary, Vieques fish assessment. Edison, NJ: Environmental Response Team Center (ERTC).

US Food and Drug Administration. 1993. Guidance document for arsenic (or cadmium/chromium/lead/nickel) in shellfish. Washington DC: Department of Health and Human Services.

US Food and Drug Administration. 1996. Fish, shellfish, crustaceans and other aquatic animals - fresh, frozen, or processed - methyl mercury (CPG 7108.07). Sec. 540.600 (rev 3/95). Compliance Policy Guides, August 1996. 237. Washington, DC: Department of Health and Human Services.

Webster. 1994. Webster=s II New Riverside University Dictionary. Boston/New York: Houghton Mifflin Company.

Report Prepared by:

Gregory M. Zarus
Atmospheric Scientist
Exposure Investigations Section
Exposure Investigations and Consultation Branch
Division of Health Assessment and Consultation
Agency for Toxic Substances and Disease Registry

Reviewers

Allan Roberson
Toxicologist
Division of Health Assessment and Consultation
Agency for Toxic Substances and Disease Registry

Kenneth Orloff
Senior Toxicologist
Exposure Investigations Section
Exposure Investigations and Consultation Branch
Division of Health Assessment and Consultation
Agency for Toxic Substances and Disease Registry

Special Acknowledgment:

ATSDR appreciates the dedication and hard work of Dan Cooke (Lockheed Martin/REAC) for the field data collection and Michelle Arbogast (Eastern Research Group) for the scientific research, advice and planning.

Approve:

________________________________ ____

Susan Metcalf, Section Chief, Date

________________________________ ____

John Abraham, Chief, DHAC, EICB Date

Appendix A
Tissue Preparation

Fish Tissue Preparation

Fish were processed according to Standard Operating Procedures for Fish Handling and Processing (USEPA 1990a). Each fish was weighed, measured for total length, and examined both externally and internally for obvious abnormalities. Fish were filleted, taking care not to include any portion of the meat that had been damaged by the spear, and a sample of the fillet was wrapped in plastic wrap, placed in a sealable plastic bag, and placed on wet ice until it could be frozen. Fish tissues were shipped on dry ice, via overnight delivery to a laboratory for analysis of target analyte list (TAL) metals, explosive compounds, percent lipids, and percent moisture.

Invertebrate Tissue Preparation

Each lobster was weighed, measured, and observed externally for obvious abnormalities. The meat sample (Alobster tail@) was removed, wrapped in plastic wrap, placed in a plastic bag, and placed on wet ice until it could be frozen.

Each conch was weighed, measured, and observed externally and internally. The conch meat was separated and wrapped in plastic, placed in a plastic bag, and placed on wet ice until it could be frozen.

The meat from several blue land crabs was picked (male and female crabs), wrapped in plastic, placed in a plastic bag, and placed on wet ice until it could be frozen (<6 hours).

All fiddler crabs were weighed together and notes were made of obvious external abnormalities. The composite sample of crabs was placed in a plastic bag, and placed on wet ice until it could be frozen (<6 hours).

All tissue samples were shipped on dry ice, via overnight delivery to the subcontracted laboratory. Tissues were analyzed for TAL metals, explosive compounds, percent lipids, and percent moisture.

Mercury Analysis

In fish tissue, mercury is present predominantly (about 85%) as methyl mercury—the more toxic form (Jones and Slotten 1996). Thus using total mercury is protective of health. Still, the methyl mercury on a few of the market samples was analyzed to check if this assumption is appropriate. One specimen of each of the four species purchased from the fish market (*red hind*, *white grunt*, *yellowtail snapper*, and *spiny lobster*) was submitted for separate methyl mercury analysis.

Appendix B
Discussion of Statistical Data

Statistical Analysis of COI Tissue Concentrations

Statistical analyses were performed to find if there were any locations where the concentrations were higher. When it can be determined that one location has higher measurements, it sometimes suggests a source of contamination. Statistical analysis uses the term *Asignificant@* quantitatively instead of the qualitative definition found in Webster=s Dictionary (Webster 1994). When scientists say that the levels at A are *significantly higher* than the levels at B, it means that, on average, the levels at A are higher than at B. It does not mean that the levels at A are notably higher than at B, and it does not tell us how much higher. Thus the original data must be examined as well as the statistical analysis of that data, and judgement used to see if there is anything unusual.

Explosive Compounds

Because explosive compounds were not found in the tissue samples, no statistical analyses were necessary.

Methyl Mercury

Because only a single individual from each of four species was analyzed for methyl mercury, and all were collected from the fish market (Location 5), no statistical analyses were performed.

TAL Metals

Statistical analyses of tissue metals were conducted to determine if Locations 1, 2, 3, 4, and 5 were significantly different from the reference area, Location 6. Tissue from groupers, grunts, parrotfish, snappers, conch, and land crabs were evaluated separately (fiddler crabs, goatfish and lobster were omitted because of limited sample numbers).

Nothing immediately unusual is apparent in the statistical results. Some locations had higher metal levels in one species, but lower metal levels in another. Some species had significantly higher levels of one metal, but significantly lower levels of another.

Three general statements can be made after reviewing the statistical analysis together with the original tissue analysis: (1) metals other than arsenic are higher in the fish caught to the south of the island, (2) metals other than arsenic are lowest in the fish to the west of the island, and (3) there is no sign of localized arsenic contamination in the fish. These points are illustrated by marking the locations which had significantly high or low metal levels. On a map, the locations of each species that had significantly high or low metal values were marked. As stated above, there appeared to be no discernable pattern. Nevertheless, after removing the arsenic data, a pattern did emerge.

Figure 2, shows the locations where a single species was significantly high or low.[14] Although the levels on the west side are significantly lower than elsewhere, the values are not much lower. Metals are significantly higher in more species caught to the south of the island (but not much higher than elsewhere). There is no clear indication that a contaminant source is present. Additionally, many of the metals found to be significantly higher to the south are minerals that are typically found in ocean water. Many factors could increase the presence of those metals, including increased evaporation.

On a map **(Figure 3),** the location and the species that had significant (i.e., high or low) arsenic values was marked. Because no location appears to have significantly high or low arsenic levels, there is no apparent source of arsenic.

[14] The figure is biased toward the more abundant species and gives equal weight to any measurement that was statistically significant no matter how much higher or lower they were.

Appendix C
Assumptions and Uncertainty

Assumptions

It was assumed that fish and invertebrates collected on the two Navy vessels that had been sunk as a result of their use as targets (Location 2) would have been exposed to the highest concentrations of COI, particularly because military training exercises had been held approximately 3 weeks prior to field sampling.

It was assumed that fish of closely related species (e.g., red hind, rock hind, graysby, and coney) at a particular sampling location would be similar enough in their life histories and their potential exposure to COI to be considered the same species for statistical purposes.

Fish and lobster purchased from the fish market were assumed to be representative of the fish consumed by the residents of Vieques.

Uncertainty

The sampling location west of Vieques (Location 6) was used as an uncontaminated control; however, it was under the control of the Navy for 60 years. Also, the west end of Vieques is both downwind and down current from the LIA and the town of Esperanza. It is possible that some contamination from the LIA and Esperanza could have migrated to the western side of the Island.

There is no way to tell if the contaminants associated with the fiddler crabs are from the crabs or LIA soil. When preparing the fiddler crabs for analysis, they were not rinsed free of sand and dirt. Because Navy personnel collected the crabs in the impact area, any dirt attached to the crabs was analyzed with the crabs.

Although the fish caught represented the size that most people eat, most of the specimens collected were small. Because the waters surrounding Vieques are heavily fished, field personnel were limited to collecting smaller fish, which could potentially have lower tissue contaminant levels than larger fish.

Because seasonality (water temperatures, presence or absence of target species) is less variable in the Caribbean than in temperate climates, it was not considered in the project sampling design.

No inorganic arsenic analysis was performed.

The statistical analysis of metals found in fish only indicates when the average levels in one location are higher than the average elsewhere. In any group of random samples, half of the set must be higher than the other half. When a cluster of higher concentrations occurs, it becomes likely that there is a localized phenomena that could cause the higher values. Many of the metals found to be statistically higher in the fish to the south are metals common in seawater (e.g., sodium, magnesium, calcium, and selenium). An increase of these minerals in the tissues could be caused by many phenomena, including increased rain to the west, increased evaporation to the south, more fresh water sources to the west, or simply by chance.