

Section 12

Toxicity Testing and Benthic Community Survey

This section summarizes the data generated from sediment and porewater toxicity tests in support of a remedial investigation of the Calcasieu Estuary in Lake Charles, Louisiana. Analyses conducted on sediments included:

- Whole sediment toxicity tests:
 - 10-d and 28-d whole-sediment toxicity tests with the amphipod *Hyalella azteca*
 - 10-d whole-sediment toxicity tests with the amphipod *Ampelisca abdita*
 - 28-d sediment bioaccumulation tests with the polychaete *Nereis virens*
 - Solid-phase Microtox® toxicity tests with *Vibrio fisheri*
- Porewater toxicity tests:
 - Sea lettuce or macroalga *Ulva fasciata thalli*
 - Sea urchin *Arbacia punctulata*
 - Redfish embryos *Sciaenops ocellatus*
- Benthic community assessments
- Toxicity Identification Evaluation

A total of 100 sediment samples were collected from the Calcasieu Estuary in November and December of 2000 (Figures 4-7 through 4-9). A more comprehensive interpretation of these tests can be found in the *Calcasieu Estuary Baseline Ecological Risk Assessment* (CDM 2002c).

Sediment is a major repository for many of the more persistent chemicals that are introduced into surface waters and provides habitat for many aquatic organisms. In the aquatic environment, most anthropogenic chemicals and waste materials, including toxic organic and inorganic chemicals, eventually accumulate in sediment. Mounting evidence exists of environmental degradation in areas where EPA water quality criteria are not exceeded, yet organisms in or near sediments are adversely affected (EPA 2000). Although certain chemicals are highly sorbed to sediment, these compounds may still be available to the biota. Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain.

Concentrations of contaminants in sediment may be several orders of magnitude higher than in the overlying water. However, bulk sediment concentrations may not always be strongly correlated to bioavailability. Because relationships between concentrations of contaminants in sediment and their bioavailability are poorly understood, determining effects of contaminants in sediment on aquatic organisms requires controlled toxicity and bioaccumulation tests and measures of effects on benthic communities inhabiting sediments (Ingersoll et al 1997).

The testing was explicitly designed to provide the information that is required to evaluate the risks to sediment-dwelling organisms that are associated with exposure to contaminated sediments. These goals were supported in two ways. First, the results will provide information of whole-sediment and porewater toxicity on benthic invertebrate community status, both of which support direct evaluations of the effects of contaminated sediments on benthic organisms. In addition, they will provide the information needed to evaluate the ability of the SQGs to correctly classify sediments in the study area as toxic or not toxic. This evaluation of predictive ability is essential to the overall ERA process because sediment chemistry will be used as a primary indicator of the potential for adverse effects on sediment-dwelling organisms. Therefore, demonstrating the site-specific applicability of the SQGs is of critical importance to the overall ecological risk assessment.

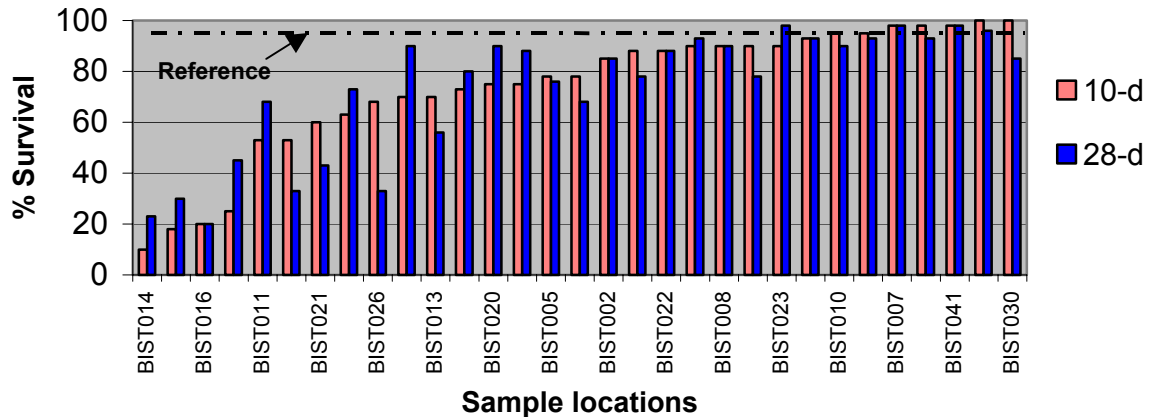
12.1 Whole-Sediment *Hyalella Azteca* Toxicity Tests

Whole-sediment toxicity tests were conducted on 100 samples with the amphipod *Hyalella azteca* following procedures outlined in ASTM (2000), EPA (2000), and Ingersoll *et al.* (1997). Exposures were conducted for 10 and 28 days, and endpoints measured at the end of these exposures included survival and length of amphipods.

Mean survival of amphipods in the control sediment for both the 10- and 28-d tests was at or above 92 percent for the first and second batches of samples. Therefore, these tests met the acceptability criteria recommended in ASTM (2000) and EPA (2000).

Wide ranges in mean survival and length of amphipods were observed with exposure to the Calcasieu sediments in the 10- and 28-d tests. In both the 10- and 28-d tests, survival of amphipods exposed to Calcasieu sediments ranged from 0 to 100 percent. Survival was reduced in comparison to reference by 20 percent of the control response in 29 percent of the Calcasieu sediments in the 10-d test and in 22 percent of the Calcasieu sediments in the 28-d test. Exhibit 12-1 illustrates a comparison of the 10-d and 28-d tests for Bayou d'Inde sediments.

Exhibit 12-1 10-d and 28-d Bayou d'Inde whole-sediment toxicity test with *Hyaella azteca* in comparison to reference



12.2 Whole-Sediment Toxicity Test Using *Ampelisca Abdita*

Amphipods were exposed to test sediments for 10 days under static conditions, following ASTM procedures. Exhibit 12-2 illustrates the observed magnitude of toxicity for the 10-d test for *Ampelisca abdita*. The results were then put into three categories, low risk, indeterminate risk, and high risk.

Exhibit 12-2 Summary of Observed Magnitude on the Survival of *Ampelisca abdita*

Area (No. of tests)	Low Risk	Indeterminate Risk	High Risk
	Survival > 55.5 percent	Survival 49.4 – 55.5 percent	Survival < 49.4 percent
Bayou d'Inde (31)	3	7	21
Upper Calcasieu (29)	16	4	9
Lower Calcasieu (15)	8	3	4
Reference Areas (14)	14	0	0
Totals (89)	41	14	34

These categories are defined as:

- **Low Risk** – Survival > 55 percent – effects that were observed or predicted to occur within a sample, reach, or an area that were similar to those of the reference areas
- **Indeterminate Risk** – Survival 49.4 percent – 55.5 percent– effects that were observed or predicted to occur within a sample, a reach, or area that were indeterminately higher in frequency or magnitude than those of the reference areas

- **High Risk** – Survival < 49.4 percent – effects that were observed or predicted to occur within a sample, a reach, or areas that were substantially higher than those for the selected reference area

The exhibit shows that out of 100 percent of the samples tested, 46 percent of the samples tested had a survival rate of greater than 55 percent, with a majority of the survival occurring in the reference area and Upper Calcasieu; 16 percent had a survival of 49.4 to 55 percent, and 38 percent of the samples demonstrated a survival rate of less than 49.4 percent, with a majority of this occurring in Bayou d’Inde (21 out of 31). A summary of the test organism survival data is presented in Appendix I.

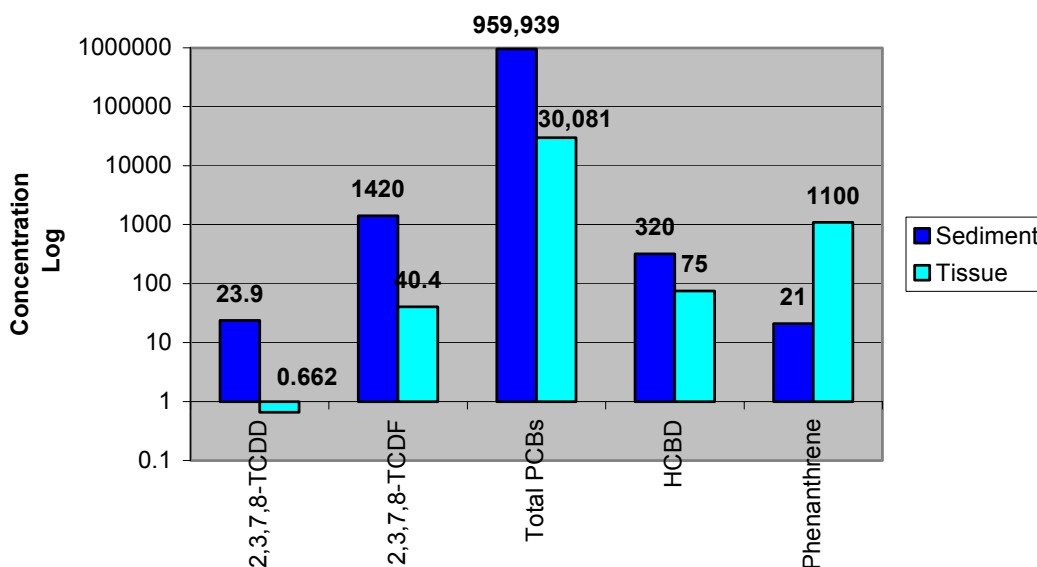
12.3 Bioaccumulation Tests Using *Nereis Virens*

Bioaccumulation is the net accumulation of a substance by an organism as a result of uptake from an environmental source. A 28-d bioaccumulation test was conducted with site sediment from 12 locations (Figures 4-7 through 4-9) within the Calcasieu Estuary and the reference areas using the polychaete *Nereis virens* as the test organism. The polychaetes were allowed to live in 2 cms of sediment for 28 days. After 28 days of exposure, the test organisms were removed from the sediment and allowed to depurate in clean seawater for 24 hours. After depuration, organisms from each replicate were rinsed in deionized water, placed into glass sample jars, and stored in a freezer at $-10 \pm 2^{\circ}\text{C}$. Frozen *Nereis virens* tissues were shipped to American Analytical and Technical Services, Inc., for chemical analyses.

Survival data for the *Nereis virens* in the bioaccumulation tests in the laboratory control sediment were 99 percent. Survival of *Nereis virens* in the site sediments ranged from 87 percent (sample station 00BI2-ST030-NSD-010) to 98 percent. Exhibits 12-3 through 12-7 show a summary of COPCs detected from the chemical analysis of the test organisms and their associated sediment and porewater sample analysis.

Bioaccumulation tests were conducted at six whole sediment sample locations in Bayou d’Inde. COPCs detected in the test organisms and sediments from Bayou d’Inde were total PCB congeners, dioxin/furans, and SVOCs. Sample location BI027 had the highest detections in test organisms for total PCB congeners (30,081 ng/kg); dioxin/furans; 2,3,7,8-TCDD (0.662 pg/g); and 2,3,7,8-TCDF (40.4 pg/g). Sediment analyzed for the same location had detects for total PCB congeners (959,939 ng/kg); 2,3,7,8-TCDD (23.9 pg/g); and 2,3,7,8-TCDF (1420 pg/g). Location BIST030 detected phenanthrene in tissue and sediment of 1,100 and 21 $\mu\text{g}/\text{kg}$, respectively. Sample location BIST011 detected HCBd 75 $\mu\text{g}/\text{kg}$ in tissue and 320 $\mu\text{g}/\text{kg}$ in sediments. Exhibit 12-3 illustrates a comparison of the COPCs detected in tissue and sediment from the same locations.

Exhibit 12-3 COPCs Detected in Bayou d'Inde Bioaccumulation Test Organisms



Metals detected in test organism were all within the range of metals detected in tissue from the reference areas. Summary tables showing all results by sample location can be found in Appendix I.

Exhibit 12-4 COPCs Detected in Bayou Verdine Bioaccumulation Test Organisms

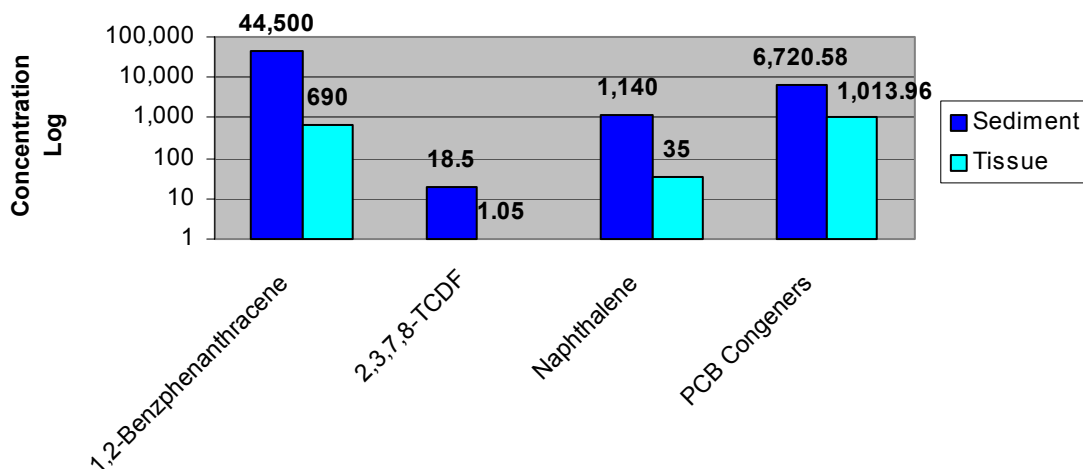


Exhibit 12-4 shows the COPCs detected in the test organisms from Bayou Verdine at sample location BV005. The bioaccumulation test was only performed at one whole sediment sample location for Bayou Verdine but with four replicate analyses. Results from these four replicates were averaged and are illustrated in Exhibit 12-4. COPCs

detected in the test organism tissue and sediment includes PCB congeners (1,013.956 and 6,720 ng/kg); 2,3,7,8-TCDF (1.05 and 18.5 pg/g); naphthalene (35 and 1,140 µg/kg); 1,2-benzphenanthracene (690 and 44,500 µg/kg); and HCDB (90 µg/kg) in tissue only. Metals detected in test organism tissue were all within the range of metals detected in the test organisms from the reference area.

Exhibit 12-5 COPCs Detected in Lower Calcasieu Bioaccumulation Test Organisms

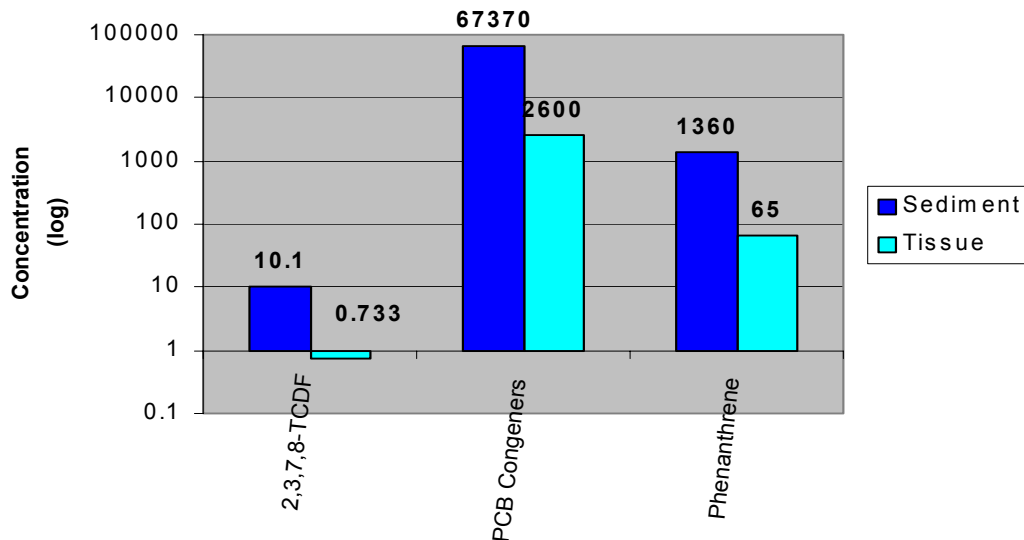
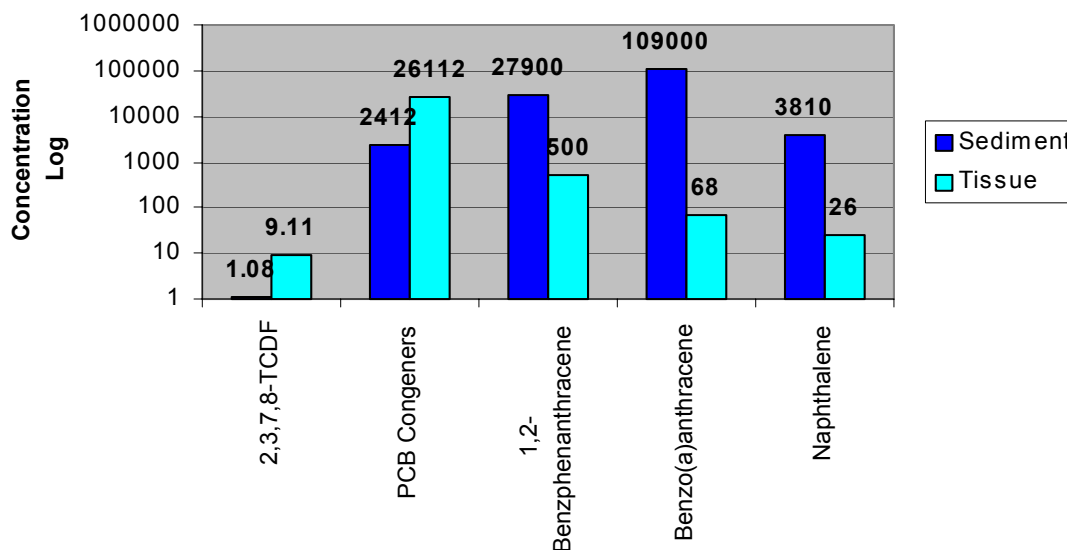


Exhibit 12-5 shows the COPCs detected in the test organism and whole sediments collected from Lower Calcasieu. The bioaccumulation test was conducted at only one whole sediment sample location in Lower Calcasieu, LC006, with four replicate analyses. The four replicates were averaged and are illustrated in Exhibit 12-5. COPCs detected in the test organism tissue and sediment includes total PCB congeners (2,600 and 67,370 ng/kg); 2,3,7,8-TCDF (0.733 and 10.1 pg/g); phenanthrene (65 and 1360 µg/kg); and HCBD (140 µg/kg). HCBD was detected in the tissue sample only due to higher detection limits for the sediment sample. Metals detected were all within the range of metals detected in the reference area test organisms.

Exhibit 12-6 shows the COPCs detected in whole sediment collected from Upper Calcasieu. The bioaccumulation tests were conducted from two whole sediment locations, UC011 and UC028, with four replicate analyses from each sample. COPCs detected in the test organism and sediment included total PCB congeners (2,412 and 26,112 ng/kg); 2,3,7,8-TCDF (1.08 and 9.11 pg/g); naphthalene (26 and 3,810 µg/kg); benzo(a)anthracene (68 and 109,000 µg/kg); 1,2 benzphenanthracene (500 and 27,900 µg/kg); and HCBD (84 µg/kg) in tissue only. Metals detected were all within the range of metals detected in the reference area test organisms.

Exhibit 12-6 COPCs Detected in Upper Calcasieu Bioaccumulation Test Organisms



COPCs detected in whole sediment from two whole sediment sample locations in the reference areas are at locations SN006 and SN011. COPCs detected at these locations in tissue included total PCB congeners (3,009 ng/kg) and 2,3,7,8-TCDF (0.388 pg/g) and in sediment at these locations 162,498 ng/kg and 5.91 pg/g.

The detections in tissue of our test organisms, with a 28-d test of COPCs, demonstrate the ability of these compounds to bioaccumulate.

12.4 Solid-Phase Microtox® Toxicity Tests

The Microtox® Solid-Phase Toxicity test (SPT test) was conducted on 100 whole sediment samples collected during the Phase II sampling. The SPT test determines the bioavailability of chemicals in sediment by exposing bioluminescent bacteria (*Vibrio fischeri*; B-NRL 1117, Azur Environmental, Carlsbad, CA) directly to sediment suspended in solution for a period of 25 minutes (Johnson 1998; Johnson and Long 1998). A log-linear model was then used to calculate EC-50 values.

The bioluminescence of bacteria at the start of the exposures was always within the acceptability limits for the test (90 to 110 percent in the control). Therefore, the Microtox® tests met the acceptability criteria recommended in Johnson and Long (1998).

A wide range in the EC50 values was observed with exposure to the Calcasieu sediments in the Microtox® test (0.15 to 37). The results were put into three

categories, low risk, indeterminate risk, and high risk. These categories are defined as:

- **Low Risk** – EC-50 less than 1.12 – effects that were observed or predicted to occur within a sample, reach, or an area that were similar to those of the reference areas
- **Indeterminate Risk** – EC-50 between 1.12 and 1.26 – effects that were observed or predicted to occur within a sample, a reach, or area that were moderately higher in frequency or magnitude than those of the reference areas
- **High Risk** – EC-50 greater than 1.26 – effects that were observed or predicted to occur within a sample, a reach, or areas that were substantially higher than those for the selected reference area

Exhibit 12-7 Microtox® EC-50 Data Summary

Area (No. of Samples)	Low < 1.12	Indeterminate 1.12 – 1.26	High > 1.26
Bayou d’Inde (32)	26	0	5
Bayou Verdine (10)	6	0	4
Upper Calcasieu (29)	26	0	3
Lower Calcasieu (15)	12	0	3
Reference (15)	14	0	0

As illustrated in Exhibit 12-7, the results indicate that exposure to whole sediments from the Calcasieu estuary poses variable risks to the microbial community, with the highest posed to the microbial community in Bayou Verdine, followed by Bayou d’Inde, and Lower Calcasieu. (CDM 2002c)

12.5 Porewater Toxicity Testing

Fifty of the 100 stations were selected for porewater toxicity evaluation to determine if there were adverse effects to survival, growth, and reproduction of aquatic plants, benthic invertebrate, and benthic or pelagic fish associated with porewater from contaminated sediments from the Calcasieu Estuary. The three tests that were conducted and their endpoints included:

- **Porewater toxicity testing with Algal zoospores** – 96-hour (h) porewater toxicity test determined with Algal zoospore using sea lettuce, *Ulva fasciata, thalli*; endpoint: germination or growth
- **Porewater toxicity testing with sea urchins** – 30-minute or 48-h toxicity test determined using the sea urchin *Arbacia punctulata*; endpoint: fertilization test and the embryological development
- **Porewater toxicity testing with redfish embryos** – 48-h porewater toxicity tests with redfish (*Sciaenops ocellatus*) embryos; endpoint: hatching success and survival

Exhibit 12-8 - Observed incidence of toxicity (IOT) summary to benthic invertebrates, aquatic plants, and fish to porewater from contaminated sediments

Area of Concern	N	IOT Benthic (percent)	IOT Aquatic Plants (percent)	IOT Fish (percent)	Risk
Bayou d'Inde	15	13	27	0	Low
Bayou Verdine	5	NA	NA	NA	NA
Upper Calcasieu	15	7	20	0	Low
Lower Calcasieu	8	25	13	0	Low
Reference	7	0	14	0	Low

NA – Not Available

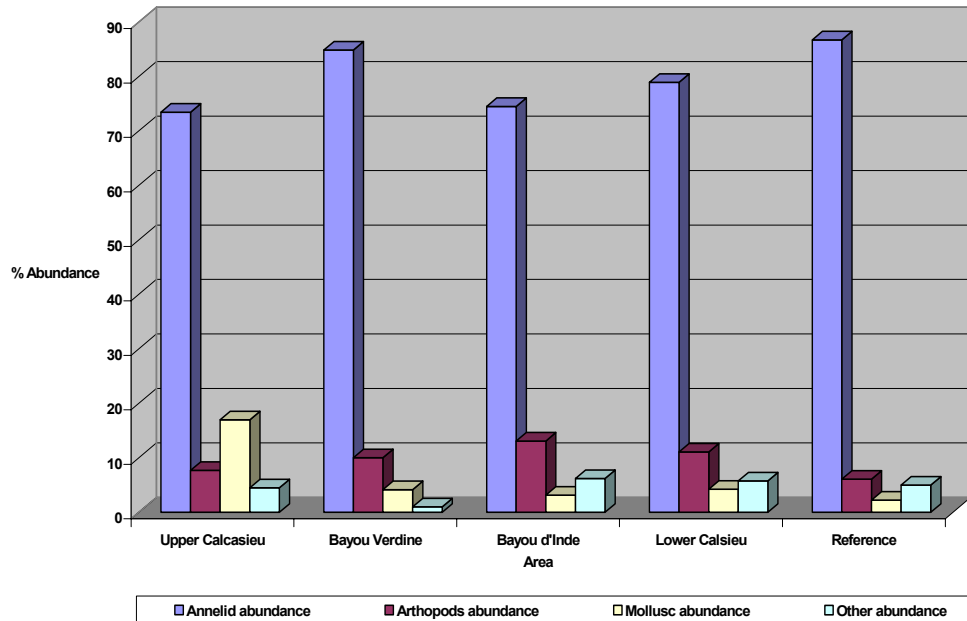
Based on the results from these tests, Exhibit 12-8 illustrates that when considered together the three lines of evidence indicate that exposure to porewater is generally not adversely affecting the survival, growth, and reproduction of aquatic plants, benthic invertebrate, and benthic or pelagic fish.

12.6 Benthic Community Survey

Estuaries of southwestern Louisiana may be characterized as vertically well mixed, with wide annual salinity fluctuations, weak tidal flushing, fine sediments (often approaching 100 percent silt/clay content), and widespread low oxygen of bottom waters during warm months (Gaston and Nasci 1988). Macrofaunal density of the region is typically modest by comparison with U.S. East-coast estuaries (e.g., Chesapeake Bay, Delaware Bay, Raritan Bay) and larger estuaries of the Gulf of Mexico (e.g., Mobile Bay, Galveston Bay). As is characteristic of macrobenthos, diversity and density values vary with numerous metrics, particularly natural environmental gradients and sediment contamination.

There were 3,408 macrobenthic organisms identified during the present study, distributed among 62 taxa. Exhibit 12-9 shows that most of the organisms were annelids (26 taxa; 77.8 percent of the specimens) and arthropods (22 taxa; 11.3 percent of the specimens). Only 11 taxa of molluscs were collected (7.7 percent of the specimens). No echinoderms were collected. Nemerteans, hydroids, and urochordates made up the remaining organisms (3.3 percent).

Exhibit 12-9 Benthic Community Survey Comparison by AOC



The species list closely resembled one reported for the region by Gaston and Nasci (1988), following their study 15 years earlier, and is characteristic of the fauna that inhabit upper reaches of Louisiana estuaries.

Abundance data for this study were comparable to values reported for the region by previous investigators (Gaston and Nasci 1988, Gaston and Young 1992). Data by station, however, ranged widely in the current study. For instance, many of the samples from Bayou d'Inde had very few organisms or very low mean total abundance per site while one station on that bayou supported 4,258 organisms/0.5 m². Gaston and Young (1992) also reported that many sites in Bayou d'Inde were devoid of macrofauna and attributed this paucity to contaminant effects. They also collected fewer organisms in Bayou d'Inde than elsewhere (Bayou Verdine and Contraband Bayou).

Densities of macrobenthos on Contraband Bayou in this study averaged 377 organisms/0.5 m². Previous studies (Gaston and Nasci 1988; Gaston and Young 1992) reported 270-macrobenthic organisms/ 0.5 m² in Contraband Bayou. Values for middle and lower Bayou d'Inde averaged 472/ 0.5 m² (previous study = 156/ 0.5 m²). Macrobenthos of lower Bayou Verdine averaged 738/ 0.5 m² (previous study = 226/ 0.5 m²). It should be mentioned that these data (current and previous studies) were not collected at the same sites.

Highest densities of macrobenthos occurred at reference sites (Choupique Bayou = 2,267/0.5 m²; Johnson Bayou = 2,651/0.5 m²) and Moss Lake (3,140/0.5 m²), Prien Lake (2,207/0.5 m²), upper Calcasieu River – Clooney Island to Coon Lake (2,059/0.5 m²), and lower Bayou d’Inde – (2,049/0.5 m²). Previous investigations of Choupique Bayou reported densities of 1,326/0.5 m² (Gaston and Nasci 1988). Gaston and Nasci sampled primarily in the salt-marsh creeks of lower Choupique Bayou, whereas the current study had more sites along intermediate marshes and adjacent to upland areas. The previous study did not sample in Johnsons Bayou, Prien Lake, or Moss Lake.

Lowest densities of macrobenthos occurred in upper Bayou d’Inde (56/0.5 m²), lower Bayou d’Inde (123 organisms/0.5 m²), lower Bayou d’Inde – lower PPG canal (130/0.5 m²), and a reference site in Grand Bayou (141/0.5 m²).

The Index of Biotic Integrity was assessed by region to make predictions of likelihood of contaminant effect on macrobenthic communities. Lowest values for the index (indicating a likelihood of contaminant effect) occurred at sites in upper Bayou d’Inde, middle Bayou d’Inde, and lower Bayou d’Inde; Bayou Olsen; and Coon Island Loop. Most of these sites supported very few organisms; and nearly all of the specimens collected were indicators of the highest levels of contamination.

Highest values for the Index of Biotic Integrity (indicative of low levels of contamination) occurred at reference sites on Johnson’s Bayou, two sites in Clooney Island Loop and single sites in Moss Lake, Prien Lake, upper Calcasieu River, lower Bayou d’Inde, and Coon Island Loop. Most of these sites supported a moderate diversity of species and relatively high abundances of macrobenthos.

Values for the Index of Biotic Integrity indicated that much of the region might be contaminated with metals and organic compounds. Most of the organisms collected were indicative of high metal and organic-contaminated sediments. Few of the organisms collected were indicators of uncontaminated conditions. The low index values for stations in close proximity to industrial development were particularly distressing. They are indicative of low abundance, low species diversity, and presence of macrobenthic species known to inhabit sediments contaminated with metals, petroleum products, and other organic pollutants.

12.7 Toxicity Identification Evaluation

Phase II sediment samples were collected from seven locations in support of EPA Region 6 TMDL study as part of the current 303(d) listing. Based on the 303(d) list, a draft TMDL is to be developed for the Calcasieu Estuary.

The purpose of the TMDL is to determine the pollutant loading that a water body can assimilate without exceeding the water quality standard for that pollutant. In support of the TMDL, a TIE was performed. The TIE method aids the TMDL determination by identifying which compound or compound groups result in toxicity to test organisms. A draft report for the TMDL study can be found in Appendix I.

The TIE methodology involved manipulation of the physical/chemical properties of sediment porewater samples to target each of the major classes of contaminant compounds. The process entails stepwise removal of each class of compounds and subsequent toxicity testing. This approach provides evidence to correlate contaminant classes with observed changes in toxic effects.

Toxicity results indicate that six of the seven sediments exhibited toxicity to both species. TIE findings for amphipods and mysids are in generally good agreement, and the results are consistent with sediment toxicity results. Exhibit 12-10 summarizes the findings from the TIE for the primary COPCs for sediment, particulate, and/or porewater.

Exhibit 12-10 Summary of Findings from the Toxicity Identification Evaluation (TIE)

Areas of Concern	COPCs
Middle Bayou d'Inde	Cu, Zn, Ni
Lower Bayou d'Inde	Cu, Zn, Ni
Lower Bayou Verdine	Cu, Zn, Ni, Pb, Cr
Prien Lake	Cu, Zn, Ni, NH ₄
Citgo Pond (Indian Marais Lagoon)	Cu, Zn, Ni, Pb, NH ₄
Bayou Olsen	Cu, Zn
Coon Island Loop (NE)	Cu, Zn, Ni, NH ₄

TIE results also indicate that specific contaminant classes caused acute toxicity and that sediment loads (via porewater flux) may be considered sources that may contribute to toxicity in the water column. The TIE study provides evidence relating site-specific contaminants and contaminant classes to observed adverse effects in benthic species. The TIE results may be applied to determine load limit requirements for protection of benthic habitat.