Application of a sediment Toxicity Identification Evaluation in support of Total Maximum Daily Load development for the Calcasieu Estuary

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A Toxicity Identification Evaluation of sediments from various reaches of the Calcasieu Estuary in Louisiana was performed to improve the technical basis for Total Maximum Daily Loads under development for this water body. The Toxicity Identification Evaluation methodology involved manipulations of the physical/chemical properties of sediment pore water samples in order to sequentially remove or bind each of the major classes of contaminant compounds and confounding factors. Toxicity tests were performed in conjunction with these manipulations to directly correlate reduction in toxicity with the specifically targeted contaminant classes. Toxicity of sediment porewaters from seven locations was evaluated using the amphipod, Ampelisca abdita and the mysid, Americamysis bahia. Toxicity Identification Evaluation results demonstrated improved survival after filtration, indicating that suspended particulates and associated contaminants were a significant source of toxicity. Subsequent treatments with column extraction and ethylene diamine tetra amino acid reduced toxicity, indicating both organics and metal constituents of pore waters as contributors to toxicity. The analysis of the filters used in the Toxicity Identification Evaluation revealed that particulate copper in the porewater was above criteria values, indicating that adverse exposure potential existed from the particulate phases, and that shifts in partitioning equilibrium could mediate dissolved-phase exposures. The identification of specific contaminant classes as well as particulate effects as sources of acute toxicity from Toxicity Identification Evaluation results indicate that in place sediment contamination as well as pore water flux and resuspension are likely contributors to habitat impairment that should be considered in Total Maximum Daily Load development.

Keywords: exposure assessment, cause-and-effect, source assessment

Introduction

Total maximum daily loads (TMDLs) for protection of waterbodies are numerical criteria that represent the upper limits of safe levels of Contaminants of Concern (CoC) inputs into water bodies from all sources that can occur without impairment of uses (i.e., fishable, swimmable). Presently, contaminated sediments are being increasingly identified as a limiting factor in the 'use attainability' of water bodies, due to in-place habitat degradation and flux to surface waters. The current and future regulatory objective is to integrate sediments in the TMDL process. However, the methodology for assigning allocations associated with specific CoCs in sediments into the TMDL process is lacking. This paper will present a Weight-of-Evidence approach including application of Toxicity Identification Evaluations (TIEs) to support development of sediment TMDLs

Aquatic Ecosystem Health & Management, 7(3):357–368, 2004. ISSN: 1463-4988 print / 1539-4077 online
DOI: 10.1080/14634980490484024

Table 1. Chronology of research and regulatory activities performed for purposes of assessment of water and sediment for potential impairment of intended uses.

Media	Time Period	Scientific Developments	Regulatory Developments
Water	1980s	Aquatic toxicity tests	Water quality criteria
	1990s	TIE/TREs	Toxicity-based, (NPDES) permitting
	2000s	Watershed Management	TMDL process
Sediment	1980s	Sediment toxicity tests	Biomonitoring programs
	1990s	Sediment porewater TIEs	Sediment quality criteria;
	2000s	Whole sediment TIEs?	Sediment TMDLs?

that are based on evidence of the sources and degrees of risks found to impair waterbodies of the Calcasieu Estuary in Louisiana, USA. The estuary is an industrialized area where several petrochemical and agrochemical plants first appeared during the early 1920s and many industrial inputs continue to the present. In March 1999, The United States Environmental Protection Agency (USEPA) began a Federal-lead Remedial Investigation and Feasibility Study (RI/FS) to evaluate the sediments in Calcasieu Estuary. The USEPA has divided the study into four areas: Bayou d'Inde, Bayou Verdine, Upper Calcasieu River (starting with the saltwater barrier) and Lower Calcasieu River (ending at Moss Lake). This TIE was conducted in conjunction with the RI/FS investigation.

Background and objectives

There has been a seesaw pattern of improved assessment followed by improved management in the development of toxicity-based approaches for water and sediment over the past three decades (Table 1). This pattern suggests that the recognition of sediment contamination as a key environmental quality issue will drive development of sediment assessment to support holistic watershed management. Currently, exceedances of water quality standards confer the water body with an 'impaired' status (303d listing) and require the development of TMDLs. In contrast, while sediment toxicity is a criterion for determining impaired status, no formal process exists to link 303d listing to sediment assessment, or vise versa. For TMDL development, sediment toxicity should, in some cases, invoke the need to reduce land-based loadings while in other cases sediment contaminant loads could represent a source to include in the total loads to the water column.

Current guidance omits the contaminated sediment link to TMDL although 25% of the 303d listings are sediment-derived. Table 2 presents a comparison of the causes cited for impairment of water and sediments respectively. The need for better understanding of causes of impairment is apparent from a review of studies conducted as to the observed causes of toxicity based on sediment/pore water results. Ho et al. (2002) reviewed a number of sediment TIE studies that demonstrate toxicity due to both organics and ammonia. This type of evidence regarding the cause(s) of failure to achieve designated uses due to degraded benthic

Table 2. Contaminant classes associated with impaired habitats in the Northeast. Data from USEPA (2001).

	Allocation of Impairment	
Cause of Impairment	Current 303d Listings*	Degraded Benthic Habitat**
Nutrients and organic enrichment	46%	NA
Pathogens	33%	NA
Metals	10%	45%
PAH/PCBs	9%	30%
Pesticides	6%	27%
Remedy:	Load reduction	Monitoring for natural attenuation; sediment remediation

*Based on water quality criteria exceedances.
**Based on SQG exceedances; NA = not assessed.

habitat suggests the need for more detailed approaches to integrate sediments into the TMDL development process.

The objective of this study was to utilize sediment TIE methods to characterize sediment toxicity sources and thereby improve the reliability of TMDLs developed for the 303d listed water bodies. In the TIE investigation, physical/chemical properties of sediment pore water samples are manipulated in order to alter or render biologically unavailable generic classes of chemicals (USEPA, 1991a). Because contaminated sediments are often toxic to aquatic organisms, fractions exhibiting toxicity reveal the nature of the toxicant(s). Depending upon the responses, the toxicant(s) can be tentatively categorized as having chemical characteristics of non-polar organics, cationic metals, or so-called 'conventional' stressors such as ammonia (USEPA, 1996). Procedures for conducting specific TIE steps developed by EPA for marine sediment pore waters using specific methodologies and QA/QC procedures form the basis for this technical approach, as outlined in the Quality Assurance/Quality Control Project Plan (SAIC, 2000).

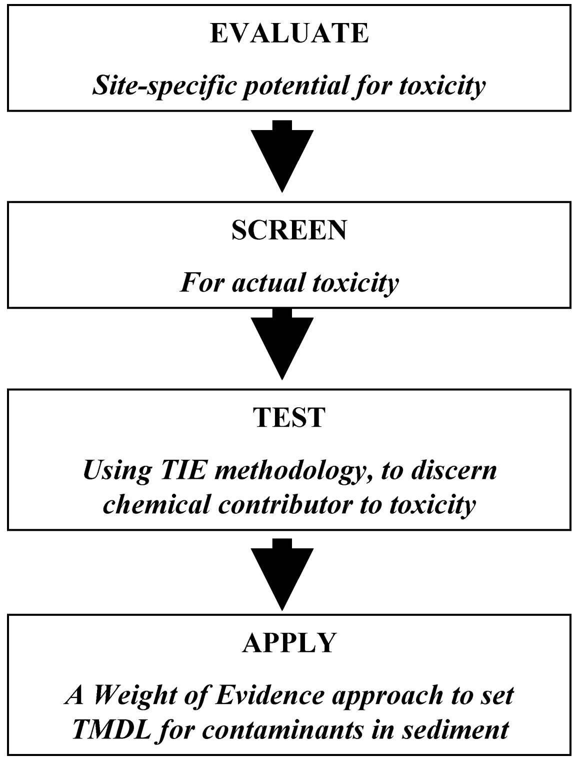

Figure 1. Procedure for using sediment toxicity identification evaluation and a Weight of Evidence approach in deriving and revising 303d listings and Total Maximum Daily Loads.

Technical approach

For the Calcasieu TMDL investigation where metals were identified as a source requiring load limits, additional investigation was warranted to evaluate sources of use impairment in sediments (i.e., degraded benthos due to chemical toxicity). Assays with multiple species, endpoints (lethal, sub-lethal), and matrices (bulk sediment and pore water) were conducted according to the screening procedure outlined in Figure 1. For the evaluation phase, data were available from existing studies, primarily the Calcasieu Superfund investigation (LDEQ and USEPA, 1997; Toxicon Environmental Sciences, 1997). The screening and test phases included the selection of specific sites for testing, field and laboratory method development and TIE testing methods performed as part of the present investigation discussed below. The development of the TMDL, including application of the TIE findings, is presented in detail in USEPA (2002), along with summary information in support of the discussion of TIE utility in TMDL development.

Selection of specific sites

Seven locations within 303d listed waters of the Calcasieu Estuary were selected for testing in the TIE study (Table 3). These stations represent a subset of locations already chosen to support an extensive program to characterize ecological risks within the waterbody (CDM, 2000). The station selection for the TIE study was based on 1) overall potential for toxicity, 2) CoC distributions that may represent different sources of toxicity, and 3) environmental conditions that may mitigate or enhance toxicity (SAIC, 2000).

Field and laboratory methods

The Superfund contractor was responsible for all field activities, including navigation, sediment collection equipment and sediment storage necessary for conducting field sampling in accordance with the Phase II Sampling and Analysis Work Plan (CDM, 2000). Analytes and methods for chemical analyses of sediment and porewater followed NOAA Status and Trends procedures (NOAA, 1998). The sediments were shipped to the TIE testing laboratory in two batches on 4–6 December 2000 and 12–14 December 2000. Extraction of pore water for the TIE was performed by centrifugation at 5000 rpm using one-liter

Table 3. Calcasieu stations selected for Toxicity Identification Evaluations.

Location	Station	Segment Court-Ordered Listed Contaminants
Lake Prien	LC2-ST003	Priority organics
Middle Bayou D'Inde	BI2-ST006	Cu, PCBs, non-priority organics, priority organics (tetrachloroethane, hexachlorobutadiene, bromoform)
Lower Bayou D'Inde	BI2-ST016	Cu, PCBs, non-priority organics, priority organics (tetrachloroethane, hexachlorobutadiene, bromoform)
Lower Bayou Verdin	BV2-ST004	Metals, priorty organics (phenols, ethylene dichloride, non-priority organics
Citgo Pond	LC2-ST007	Not listed
Bayou Olsen	LC2-ST009	Cu, priority organics
Coon Island Loop	NE UC2-ST021	Cu, Hg, priority organics, metals, ammonia

centrifuge bottles. Control and dilution water was collected from surface waters at the amphipod collection site.

Toxicity Identification Evaluation

Science Applications International Corporation (SAIC) has modified the USEPA's parallel TIE testing approach by applying sequential testing of fractions and documentation of cumulative removal up to and including the production of completely non-toxic samples (Figure 2). Using the sequential approach, absence of residual toxicity allows the opportunity to demonstrate that all the relevant chemical exposures in a sample have been addressed (SAIC 2001, 2002a,b, 2003). The TIE characterization consisted of the following characterization steps or tiers: 1a) Baseline Toxicity Test, 1b) Filtered Sample Toxicity, 2) C18 column extraction, 3) sodium thiosulfate (STS), 4) ethylene diamine tetraacetic acid (EDTA), and 5) graduated pH. Guidelines for conduct of specific TIE methods were followed as presented in USEPA (1991a,b, 1996). For each treatment, dilutions were prepared to generate a series of four test concentrations (10%, 25%, 50%, and 100% pore water). Water quality measurements (temperature and pH) were recorded for each sample prior to distribution into the dilution series. Upon test initiation and termination, pH and dissolved oxygen were measured in each of the water quality replicates, and in an animal exposure replicate. Temperature was monitored daily. Animal survival was monitored at daily intervals for the duration of the 96-hour exposures. Total ammonia in pore water was measured using an ion-specific probe. Unionized ammonia was calculated using the formulas applied by Hampson (1977) to represent the more toxic form.

Results

Screening toxicity results

Table 4 reports results of the Superfund testing program, including bulk sediment tests with the freshwater amphipod "*Hyalella azteca*" and pore water tests using Microtox and sea urchins (*Arbacia punctulata*). Table 4 also includes results from untreated pore water tests used in the present TIE study, including the marine amphipod *Ampelisca abdita* and mysid *Americamysis bahia*. Sediment test results show toxicity ($<80\%$ survival) in four of the seven stations (UC2-ST003, LC2-ST016, LC2-ST004, and LC2-ST007), while Microtox tests indicate all samples are toxic ($LC_{50} < 25\%$). Porewater tests with sea urchins indicate reduced fertilization in Stations LC2-ST016, LC2-ST004, LC2-ST007 and UC2-ST021, and complete toxicity to larval development (0% normal development) in all samples. In the present TIE study with amphipods and mysids, all pore water samples except the one from Bayou Olsen (LC2-ST009) were toxic as untreated pore water and were therefore included in the TIE.

Toxicity Identification Evaluation

TIE manipulations and toxicity testing with the six toxic pore waters were completed with both the amphipod and the mysid. For this paper, results are provided in detail only for the amphipod; the findings for the mysid were generally concordant with the amphipod results (SAIC, 2002a) and hence are used to support the overall weight-of-evidence for toxicity characterization. Tests with *Ampelisca* yielded acceptable performance control survival above 87% in all of the primary TIE treatments. The pH performance control

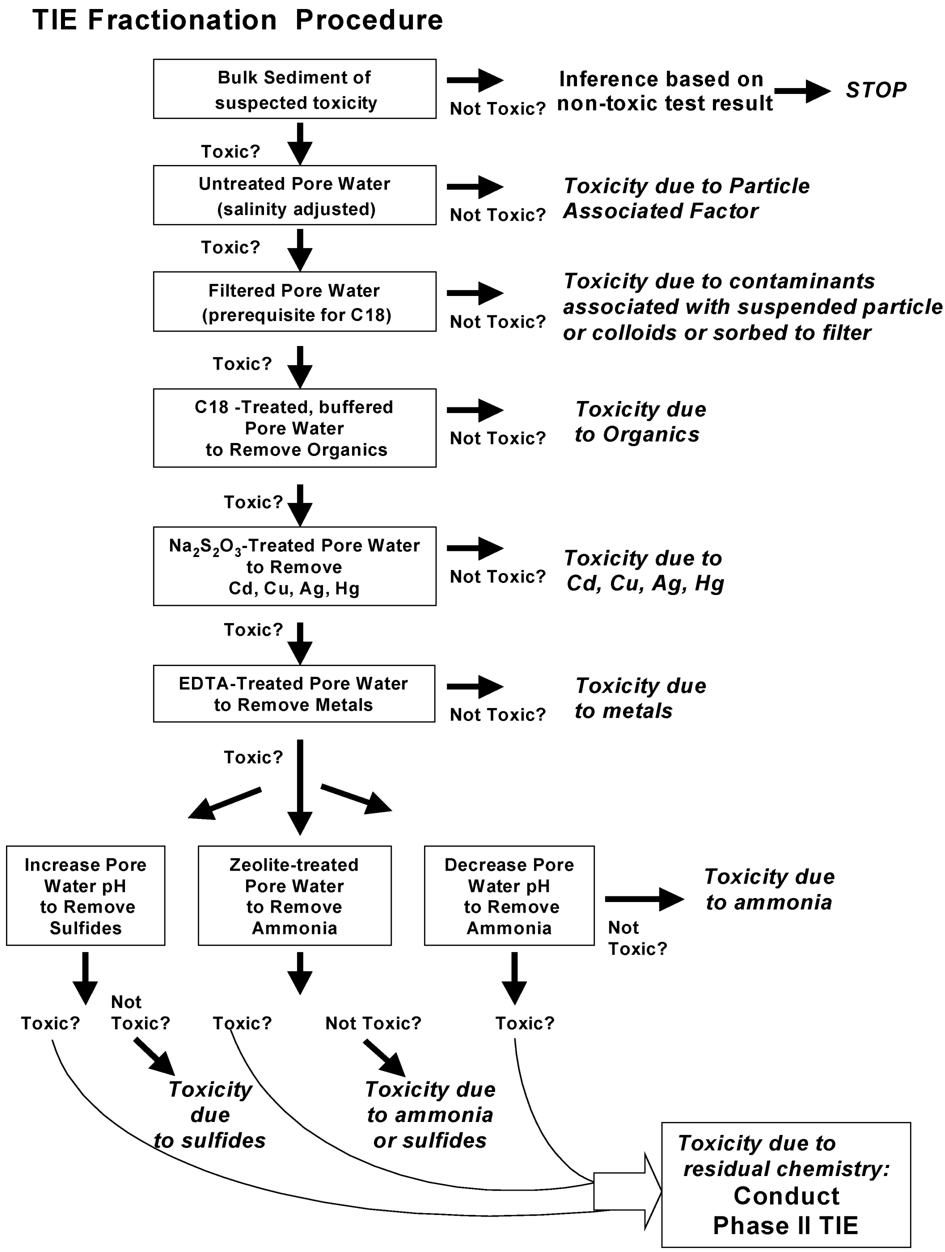

Figure 2. Sequential fractionation procedure for pore water Toxicity Identification Evaluation (TIE).

results used to assess toxicity from confounding factors (i.e., ammonia, sulfides) were below optimal levels (ASTM, 1988) as survival was reduced to 67–80% for amphipods. It is postulated that pH-related disequilibria is likely to have caused the observed mortality (also observed in mysids) as gradual acclimation was not applied in order to test sensitive early stages of mysids, and to minimize holding times for amphipods. Because of the poor performance control results, findings for the pH tests are not presented.

The interpretation of TIE toxicity responses as presented below was based on both the observed magnitude of the toxicity in the treated sample as well as the relative change in toxicity from the previous sample result in the TIE sequence. It is noted that lower dilutions (e.g., 10%) represent less concentrated samples, and

Table 4. Comparison of mean results from toxicity tests conducted with *Arbacia punctulata* (sea urchin), the amphipods *Hyalella azteca* and *Ampelisca abdita*, and the mysid *Americamysis bahia* (mysid) on Calcasieu Estuary samples.

		Sediment Tests[2]		Pore Water Tests[1]		TIE Tests	
Sample Name	Sample Identification	*Hyalella* 10-Day Whole Sediment Test (% Survival)	Solid-Phase Microtox Test (EC_{50})	Sea Urchin Fertilization Test (% Fertilized)	Sea Urchin Embryological Development Test (% Normal Dev't)	*Ampelisca* Porewater Test (% Survival)	Mysid Porewater Test (% Survival)
Lake Prien	LC-ST003	78	2	78	0	26.7*	0*
Middle Bayou d'Inde	BI-ST006	100	5	84	0	0*	0*
Lower Bayou d'Inde	BI-ST016	20	10	9	0	0*	0*
Lower Bayou Verdine	BV-ST004	15	0	2	0	0*	0*
Citgo Pond	LC-ST007	73	14	6	0	0*	0*
Bayou Olsen	LC-ST009	93	2	87	0	93	87
Coon Island Loop NE	UC-ST021	90	0	44	0	60*	0*

[1]Data source: Carr et al., 2001.
[2]Data source: MacDonald Environmental Sciences, 2001.
*Statistically different (a = 0.05) compared to the control data.

Table 5. Summary of toxicity testing results for amphipods for each Calcasieu sampling location and Toxicity Identification Evaluation treatments. Bold values: Statistically different from Performance Control Samples >80% = non-toxic (NT); Underline values: statistically different from prior treatment response; ORG = Organic, PAR = Particulate.

	TIE Treatment Results					
	Ampelisca abdita (% Survival)					
Station-Dilution	Untreated	Filtered	C-18	Na_2SO_3	EDTA	Inferred Toxicity Source[1]
LC2-ST003 - 10	100					NT
LC2-ST003 - 25	80					NT
LC2-ST003 - 50	67	86.7				PAR
LC2-ST003 - 100	27	**67.8**	100.0			PAR, ORG
B12-ST006 - 10	87					NT
B12-ST006 - 25	73	100				PAR
B12-ST006 - 50	27	93.3				PAR
B12-ST006 - 100	0	**13.3**	**35.6**	**53.3**	**33.3**	PAR, ORG
B12-ST016 - 10	93					NT
B12-ST016 - 25	80					NT
B12-ST016 - 50	7	93.3				PAR
B12-ST016 - 100	0	**5.6**	**46.7**	**66.7**	80.0	PAR, ORG, MET
BV2-ST004 - 10	27	93.3				PAR
BV2-ST004 - 25	0	86.7				PAR
BV2-ST004 - 50	0	**6.7**	**6.7**	**6.7**	**13.3**	PAR, UNK
BV2-ST004 - 100	0	**0.0**	**0.0**	**0.0**	**0.0**	PAR, UNK
LC2-ST007 - 10	67	93.3				PAR
LC2-ST007 - 25	40	86.7				PAR
LC2-ST007 - 50	0	60.0	100.0			PAR, ORG
LC2-ST007 - 100	0	**0.0**	**20.0**	**6.7**	**53.3**	PAR, ORG, MET
LC2-ST009 - 10	93					NT
LC2-ST009 - 25	100					NT
LC2-ST009 - 50	93					NT
LC2-ST009 - 100	93					NT
UC2-ST021 - 10	87					NT
UC2-ST021 - 25	80					NT
UC2-ST021 - 50	87					NT
UC2-ST021 - 100	60	**40.0**	**55.6**	**53.3**	**46.7**	UNK
PC-100	100	88.9	100	86.7	93.3	

[1]Inferred toxicity source incorporates findings from both lower dilution and present dilution (e.g., cumulative effects).

hence should be expected to cause less toxicity (i.e., higher survival). For the purpose of the TIE, the lower dilutions contain only the more potent constituents that contribute to toxicity, and thus the toxicity reduction is more readily observed through the TIE process.

A summary of toxicity testing results for amphipods for each sampling location and TIE treatment is presented in Table 5. To simplify the data presentation, bolded values are used to represent results that are statistically significantly different from the control, while underlined values indicate a statistically significant difference from the preceding TIE treatment results. In addition, the displayed data only includes samples that remained toxic (i.e., <80% survival) after the prior treatment. Finally, an interpretive summary as to the source of toxicity is provided for each dilution (PAR = particulate, ORG = organic, MET = metal, UNK = unknown). Results are discussed by sampling locations below:

For Lake Prien (LC2-ST003), amphipods showed improved survival in 50% and 100% dilutions after filtration, indicating that particulate-associated contaminants were a significant source of toxicity. Improved survival was again observed for the C18 treatment, suggesting an organic contaminant as a source of toxicity. For Middle Bayou D'Inde (BI2-ST006), amphipods showed improved survival in the three upper (25%, 50% and 100%) dilutions after filtration, indicating that particulate-associated contaminants were a significant source of toxicity. Slightly improved survival was again observed in the highest dilution for the C18 treatment, suggesting an organic contaminant as a source of toxicity. Toxicity in the highest dilution was not further resolved and thus suggests an untreated source of chemical exposure still exists in the sample.

In the Lower Bayou D'Inde (BI2-ST016) sample, amphipod survival was greatly improved survival with filtration in the 50% dilution and a slight response in the 100% dilution. Subsequent treatment of the latter sample revealed both organics and metal constituents as contributors to toxicity. As with the previous samples, the Lower Bayou Verdine (BV2-ST004) toxicity was greatly improved survival with filtration in the two lower dilutions and a slight response in the 50% dilution. Subsequent treatment of the latter sample and the highest dilution did not improve survival. Thus while a particulate source of chemical toxicity was confirmed, untreated source of chemical exposure still exists in the sample; high 'salinity' and extremely high levels of calcium (Ho, 2001) indicated the presence of an ionic imbalance that would not be effectively treated with the TIE methods.

For Citgo Pond (LC2-ST007), amphipods showed improved survival in the three lower dilutions (10%, 25% and 50%) after filtration, indicating that particulate-associated contaminants were a significant source of toxicity prior to the C18 column extraction treatment. Improved survival was again observed in the 50% dilution (and perhaps the 100% dilution, though this latter was not statistically significant), suggesting an organic contaminant as a source of toxicity. Toxicity was further reduced in the highest dilution by the EDTA treatment, suggesting metals as a source of toxicity. Still, the toxicity was not completely removed such that an untreated source of chemical exposure still exists in the sample. Finally, for the Coon Island Loop NE (UC2-ST021) location, only the highest (100%) dilution was toxic to amphipods, and TIE treatments were ineffective in resolving the source. In contrast, mysids were more sensitive to constituents in the sample (data presented in SAIC, 2002a) with improved survival with filtration in the lower dilutions. Further improvement with column extraction treatment was also noted for mysids in the 25% dilution indicating organics as a source of toxicity.

Chemical exposure characterization

Chemical concentrations of metals, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides measured in porewater and sediment were compared to American Water Quality Criteria (AWQC) (metals) and screening criteria derived from sediment benchmarks via equilibrium partitioning predictions (organics, DiToro et al., 1991) as an independent indicator of toxicity sources. For clarity of presentation, analytes having concentrations less than 10% of the respective benchmark values in one or more samples were excluded. As shown in Table 6, Cu concentrations in six of the TIE samples exceeded the AWQC by 20 to 130% (e.g., hazard quotients (HQs) = 1.2–2.3); the remaining sample (BI2-ST016) approached 78% of the AWQC value. Nickel exceeded AWQC at one location (BV2-ST004, HQ = 1.5). In addition, Zn was three- to twelve-fold above AWQC in six of seven samples. Concentrations of organics compared to benchmarks were generally below predicted adverse effects levels, the only exception being found for phenanthrene (BV2-ST004, HQ = 1.4) and gamma BHC (LC2-ST003, HQ = 2.0). In

Table 6. Hazard Quotients calculated for CoC concentrations in sediment pore waters of TIE sampling locations. HQ = pore water concentration/ambient water quality criteria acute value; HQ data from SAIC (2002). Only analytes for which and HQ > 1 was observed in one or more samples is shown. HQ values exceeding unity are underlined.

Class	Analyte	BI2-ST006	BI2-ST016	BV2-ST004	LC2-ST003	LC2-ST007	LC2-ST009	UC2-ST021
MET	Copper	*1.34*	0.78	*2.33*	*1.30*	*1.70*	*1.22*	*1.31*
MET	Nickel	0.08	0.15	*1.49*	0.07	0.08	0.06	0.09
MET	Zinc	*7.39*	*4.13*	*4.42*	*3.00*	*2.97*	*12.3*	0.44
PAH	Phenanthrene (L)	5.7E-3	6.1E-3	*1.37*	8.8E-3	3.7E-3	6.8E-3	0.01
PST	Gamma-BHC (Lindane)	0.76	0.56	0.56	*2.02*	0.49	0.32	1.27

addition to the above, Station BV2-ST004 had a number of PAHs that approached 10 to 50% of the benchmark, and all the samples had gamma BHC concentrations that approached 30 to 80% of the benchmark. Endrin and heptachlor epoxide may also be at levels that could contribute to toxicity in a number of the TIE samples.

Weight of evidence characterization

A summary of general findings of the TIE testing and exposure characterization is provided in Table 7. As noted in the Superfund investigation and in the present study, substantial toxicity was observed using a wide variety of test endpoints. The most sensitive endpoints included the Microtox and *Arbacia* larval development tests that displayed nearly complete adverse responses. The other Superfund study endpoints (the *Hyalella* bulk sediment test and the *Arbacia* fertilization test) as well as the present TIE endpoints (*Ampelisca* and *Americamysis* porewater tests) were in good agreement in displaying a range of toxic responses and similar results at the same locations.

The TIE process suggested one or more contaminant classes associated with toxicity in each of the six toxic Calcasieu pore waters. Overall, the principal chemical-specific TIE signal was the reduction in toxicity associated with filtration of metal-laden particulates prior to C18 treatment for removal of organics. The toxicity changes associated with C18 manipulation indicate that non-polar organics such as PAHs were substantial contributors to acute toxicity. Sodium thiosulfate (STS) reactions and EDTA chelation also consecutively reduced toxicity to lesser degrees.

The filtration-related results alone offer no clear interpretation because the filter may retain particulates and colloidal aggregates from multiple classes of contaminants, including non-polar organics and metals (Ankley et al., 1992). However, based on measured concentrations of both filtered and porewater metals, and reductions in toxicity through the three metal-modifying procedures (filtration, STS and EDTA), exposures to elevated levels of Cu and Zn are definitively indicated as causes of toxicity (Table 7). Some residual toxicity was observed in a number of samples and may be associated unionized ammonia concentrations that were above LC_{50} values; hence, ammonia may have been responsible for the observed toxicity. The high residual toxicity in the Bayou Verdine sample was most likely due to unfavorable ionic conditions that were not addressed though the TIE. When associated with elevated Ca levels, the resulting ionic imbalance deviates beyond the tolerance levels of the test species involved (Ho, 2001).

In contrast to the comparison of sediment concentrations relative to sediment benchmarks, the analysis of the filters used in the TIE revealed that particulate Cu in the porewater exceeded AWQC in all samples except for the Bayou Olsen and Coon Island Loop NE stations. The Bayou Verdine sample lost the greatest concentration of Cu to filtration (32 times the AWQC), followed by the Bayou d'Inde samples (4.6–7.3 times the CMC). This supports the conclusion that Cu partitioned considerably to both fine particulate and dissolved phases. While only the dissolved phase is generally assumed the bioavailable fraction, the filter-associated particulate metals could represent a more loosely bound phase than the metals in bulk sediment. Elevated pore water concentrations, including suspended particulate phase Cu and Zn in these samples support this hypothesis.

Sodium thiosulfate produced a moderate reduction in amphipod toxicity in the lower Bayou d'Inde sample but this was not confirmed in the corresponding mysid sample. The signals from both STS and EDTA observed with Bayou d'Inde samples are consistent with the presence of Cu^{2+} in the pore water samples. A modest reduction in sample toxicity due to EDTA treatment was also noted in the Citgo Pond (LC2-ST007) sample. This finding is consistent with chelation of remaining metals after those sequestered by the previous sodium thiosulfate treatment. Given the measured metals, Cu and Zn are both are likely candidate involved in the toxicity response to the Citgo Pond pore water. The STS concentration may have been insufficient to remove all toxic Cu, and EDTA would then serve to chelate both metals. No other samples demonstrated a strong EDTA signal. After Bayou Verdine (BV2-ST004, HQ = 2.9), Citgo Pond had the next highest non-filterable concentration of Zn (HQ = 1.7; SAIC, 2002a).

As stated above, it is uncertain how much of the filterable metal might be bioavailable, but the above results suggest that dissolved metals passing through the filter were a source of toxicity in some of the samples as noted. Reduction in toxicity due to removal of organics with C18 was observed in five of the seven pore waters, indicating that non-polar organics are also an important source of toxicity identified by the TIE. Finally, with regard to the cases of residual toxicity, ammonia was above the threshold for effects associated with unionized ammonia and, thus, was a likely a source of toxicity. Alternatively, unusual constituents such as the elevated Ca measured in the Bayou Verdine sample (not evaluated in other samples) or residual concentrations

Table 7. Summary of findings from the Calcasieu Toxicity Identification Evaluation.

Calcasieu Area	Sample ID	Toxicity Superfund[1A]	Toxicity TIE[1B]	TIE Results[2]	CoC Sediment[3]	CoC Particulate[4]	CoC Porewater[5]
Middle Bayou D'Inde	B12-ST006	Hya, Mic, Dev	Amp, Mys	FILT > STS = C18	Ni?, Zn, SEM?		Cu, Zn, NH_4
Lower Bayou D'Inde	B12-ST016	Mic, Dev	Amp	FILT > STS > C18	Ni?, Zn?, SEM?	Cu, Zn	Ni?, Zn, NH_4
Lower Bayou Verdine	BV2-ST004	Hya, Arb, Mic, Dev	Amp, Mys	FILT	Ag?, Ni, Zn?, SEM	Cr?, Cu, Pb?, Zn	Cu, Ni, Zn, PAH
Lake Prien	LC2-ST003	Hya, Arb, Mic, Dev	Amp, Mys	FILT > C18	Ni?, Zn?, g-BHC?	Cu, Zn?	Cu, Zn, g-BHC, NH_4
Citgo Pond	LC2-ST007	Hya, Arb, Mic, Dev	Amp, Mys	FILT > EDTA > C18	Ni?, Zn?, SEM?	Cu, Pb?, Zn	Cu, Zn, NH_4
Bayou Olsen	LC2-ST009	Mic, Dev	Non-Toxic	Non-Toxic	Zn	Zn?	Cu, Zn
Coon Island Loop NE	UC2-ST021	Arb, Amp, Mic, Dev	Amp, Mys	FILT > C18 > STS	Ni?, Zn?	Zn?	Cu, NH_4

[1A] Hya = *Hyalella* sediment toxicity, Arb = *Arbacia* fertilization toxicity, Mic = Microtox, Dev = *Arbacia* larval development toxicity.
[1B] Amp = *Ampelisca* pore water TIE toxicity; Mys = *Americamysis* TIE pore water toxicity.
[2] Treatments found to reduce toxicity.
[3] Analytes in sediments exceeding benchmarks, ? = potential toxicant based on conc. >10% of benchmark.
[4] Analytes on particulates in pore water exceeding benchmarks, ? = potential toxicant based on conc. >10% of benchmark.
[5] Analytes in pore water exceeding benchmarks, ? = potential toxicant based on conc. >10% of benchmark.

of measured chemicals that remained untreated might also be contributors to toxicity that was not removed by the TIE.

Conclusions

In evaluating the potential toxicity associated with chemical contamination versus confounding factors, multiple lines of evidence were reviewed, including the full toxicity results (Superfund and untreated TIE samples) and TIE signals (Filtration, STS, EDTA and C18 treatments). Hazard Quotients from dissolved porewater concentrations (HQ = measured concentration/water quality criteria value) were evaluated based on the larger Superfund co-located toxicity and chemistry database. These results suggest that the pore water concentrations are more closely correlated with sediment toxicity than were the bulk sediment concentrations. The importance of this finding to developing appropriate TMDLs for the Calcasieu Estuary is two-fold. First, the identification of specific contaminant classes that caused acute toxicity provides strong support for the association between anthropogenic sources of contamination and the need for TMDLs for those contaminants. Secondly, whether or not current sources for the toxic contaminant classes are identified, the present sediment loads should be considered as sources that may contribute to toxicity in the water column, either via pore water flux or sediment resuspension events.

The TIE derivation procedures presented herein provide valuable evidence relating site-specific contaminants and contaminant classes to observed adverse effects. While this study demonstrates the utility of using TIE methods to support TMDL decisions, there is currently no guidance or regulatory framework to include this process. Ironically, sediment toxicity is one of the criteria used to place waterbodies on the national 303(d) list of impaired waters requiring TMDLs, but without knowledge regarding the cause(s) of toxicity, it is not possible to determine which loads to restrict, and to what degree. The Water Environment Research Federation is currently sponsoring a research effort working toward appropriate guidance for using TIEs as part of a weight-of-evidence for TMDL listing, delisting and development (WERF, 2002). In the case of the Calcasieu TMDL (USEPA, 2002) the TIE findings were combined with traditional TMDL methodologies (source loads, estimation of historic loads and transport and fate models) to support, the need for metal and organic load limits for protection of benthic habitat. In the future, TIEs should be used as a critical line of evidence to establish which, if any, classes of sediment contaminants are toxic to aquatic biota, in order to focus remedial goals that will reduce risks associated with identified exposure routes.

Acknowledgements

We wish to thank the coordinators of the Calcasieu Superfund study, including John Meyer (USEPA, Region 6) and Don MacDonald (MESL). We would also like to thank Jim Biedenbach, Chris Ingersol and Ann Shortell and their teams for their timely delivery of samples and toxicity data. In addition, we thank Tom Carpenter (SAIC) for initiating the use of TIEs for the Calcasieu TMDL effort.

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