
Reducing Uncertainty in Laboratory Sediment Toxicity Tests

Health and Environmental Sciences Department
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Health and Environmental Sciences Department

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ABSTRACT

Many contaminants entering aquatic systems may accumulate in sufficient quantities in sediments to adversely impact benthic organisms. Laboratory sediment toxicity tests may be the most accurate means of determining whether or not these sediments are toxic to benthic communities. However, concerns have been expressed regarding the ability of existing laboratory sediment toxicity testing methods to accurately assess sediment toxicity. This report presents methods for improving laboratory sediment toxicity tests. A formulated reference sediment was developed that is suitable for survival, growth, and reproduction of commonly used sediment testing species. Copper sulfate was evaluated and found to be a suitable reference toxicant for sediment toxicity tests. Tolerances of *Hyaella azteca* and *Chironomus tentans* were determined for various particle size classes and organic matter content of sediments. This information can be used to reduce the likelihood of erroneously concluding that a sediment is toxic simply because the sediment is incompatible with test organisms. An evaluation of the relative sensitivities of commonly used sediment testing organisms and sublethal endpoints using a copper-contaminated sediment indicated that test duration, species selection, and test endpoint can influence test results. These data provide a means for increasing the accuracy of sediment toxicity test results.

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EXECUTIVE SUMMARY

Many consolidated sediments of aquatic systems near urban or industrial areas contain elevated concentrations of materials such as metals, organics, and pesticides that could adversely affect organisms associated with bottom sediments. Historically, most of the attention on materials in aquatic systems has focused on limnetic organisms. However, sediments are an important habitat for benthic organisms that may spend nearly their entire life cycle in intimate contact with bottom sediments and have important roles in aquatic systems where they occur. Clearly, sediments are vital components of aquatic environments, providing habitat, feeding and breeding areas for many aquatic organisms. Thus, it would be useful to be able to accurately assess the toxicity of contaminants in sediments.

Currently, there are a number of sources of information that may provide presumptive evidence of sediment toxicity and bioavailability. These include: 1) a list of contaminants in sediments with their associated concentrations, 2) survey of the total number of benthic organisms and species present, and 3) laboratory experiments. However, there are uncertainties and ambiguities associated with each of these sources of information. The presence of a material or contaminant in sediment does not definitively indicate that the sediment is toxic. Presence or absence of benthic species inhabiting a particular site is only presumptive evidence of sediment potency because such studies do not take into account physical characteristics of sediments or environmental perturbations that may render the sediment inhospitable to benthic species. Therefore, the use of laboratory experiments to assess the potency of sediments may provide the most direct evidence of sediment quality. Unfortunately, current approaches for conducting whole sediment experiments for predicting potency or bioavailability of materials in sediments have weaknesses that limit their ability to accurately assess sediment quality.

Since the potential for contaminated sediments to serve as sources or sinks for contaminants has not been sufficiently evaluated, and sediments serve as vital habitat for many organisms in aquatic systems, this research focused on evaluating critical components of laboratory experiments that may contribute to more accurate assessments of sediment quality.

The concerns and uncertainties related to laboratory experiments and sediments led to the experiments described in this report. The intent of these experiments was to help clarify questions regarding the accuracy of laboratory experiments and the ability of these experiments to predict adverse effects of sediment-associated contaminants. These results should help provide increased accuracy for laboratory experiments in predicting adverse effects of sediment-associated contaminants.

Formulated sediment was prepared to match representative natural sediments with respect to various sediment characteristics and provided a suitable substrate for toxicity testing organisms during life cycle exposures. Constituents used to prepare formulated sediment were commercially available, thus permitting widespread use of this sediment as a standard reference sediment.

In definitive sediment toxicity tests, formulated sediment, when used to dilute a copper contaminated sediment, provided predictable exposure-response curves for all organisms at all test durations. Formulated sediment provided a suitable reference and control sediment for the copper contaminated sediment, thus providing a means of accurately assessing sediment potency.

Formulated sediment was also used to successfully determine *Hyaella azteca* and *Chironomus tentans* tolerances of particle size regimes and organic matter content of sediments. *H. azteca* and *C. tentans* were tolerant of a wide range of particle size regimes and organic matter content of bottom sediments, thus making them suitable test organisms to assess sediment quality in most situations. Formulated sediments should be suitable for evaluating tolerances of other benthic toxicity testing organisms for sediment characteristics, thus reducing somewhat the risk of drawing erroneous conclusions regarding sediment toxicity.

These experiments and development efforts have provided clear evidence of the tremendous utility and potential for formulated sediments in advancing sediment quality evaluations.

Results from water-only and sediment copper toxicity experiments with test durations ranging from 48 hours to 14 days indicated differences in survival between toxicity testing organisms, with organisms listed in order of decreased sensitivity to copper: *Ceriodaphnia*

dubia, *Daphnia magna*, *Pimephales promelas*, *H. azteca*, and *C. tentans*. A threshold was observed for *C. dubia* and *D. magna* survival after 96 hours of exposure to aqueous and sediment-bound copper. For *H. azteca*, *C. tentans*, and *P. promelas*, exposure durations of at least 10 - 14 days may be required for these organisms to correctly assess copper toxicity in whole sediment and water-only exposures.

Examinations of sublethal endpoints indicated that 14 days of exposure were required to elicit a reduction in *C. dubia* reproduction, which was not observed after 7 and 10 days of exposure. Growth was not a sensitive endpoint for *P. promelas* when exposed to a copper contaminated sediment in 10 and 14 day exposures. *C. tentans* growth was more sensitive than survival during a 10 day exposure. *C. tentans* growth was considerably more sensitive than survival when exposed to copper contaminated sediment. Results from this study with a copper contaminated sediment indicated that test duration was critical in eliciting sublethal endpoints for *C. dubia* and *C. tentans*. These results helped clarify some sources of uncertainty regarding test duration and endpoints for these test organisms exposed to copper.

This study provided evidence that *C. dubia*, *D. magna*, *H. azteca*, *C. tentans*, and *P. promelas* are suitable as toxicity testing organisms in assessing sediment copper toxicity. Although *H. azteca* and *C. tentans* were generally less sensitive to copper in sediment than *C. dubia* and *D. magna*, they may be more sensitive to other sediment-sorbed contaminants. Another consideration is the degree in which an organism is associated with sediment. For example, daphnids are generally considered water column organisms, although *D. magna* graze on sediment surfaces. *H. azteca* and *C. tentans* are both considered benthic organisms, although their association with sediment differs. *H. azteca* is an epibenthic detritivore, feeding on organic material on the sediment surface and on rooted aquatic vegetation, thus is in contact with sediment and the overlying water. *C. tentans*, however, is a benthic infaunal organism, which during its larval stage, constructs a case from organic material in sediment and thus is in intimate contact with sediments. By using several organisms that represent a variety of niches, the likelihood of making an erroneous conclusion regarding sediment toxicity can be reduced.

INTRODUCTION

The use of laboratory experiments to assess the potency of sediments may provide the most direct evidence of sediment quality. Unfortunately, current approaches for conducting whole sediment experiments for predicting potency or bioavailability of materials in sediments have weaknesses that limit their ability to accurately assess sediment quality. To address these uncertainties, a series of experiments were performed: 1) development and evaluation of a formulated reference sediment; 2) examination of the tolerances of commonly used freshwater sediment testing species to sediment characteristics; 3) evaluation of copper sulfate as a sediment reference toxicant; and 4) determination of the relative sensitivities of freshwater testing organisms to a copper contaminated sediment and an evaluation of potential sublethal endpoints that can be used to evaluate sediment potency.

To develop and evaluate formulated reference sediments for use in definitive sediment toxicity testing, the rationale behind developing formulated reference sediment was reviewed and potential formulated sediment constituents and their physical and chemical properties examined. The formulated reference sediment evaluation included: 1) matching formulated reference sediment with representative freshwater sediments from the University of Mississippi Biological Field Station (UMBFS) and estuarine sediments from Horn Island, MS; 2) life-cycle experiments examining formulated reference sediment as a suitable substrate for survival and reproduction of the amphipod, *Hyaella azteca* Saussure, midge, *Chironomus tentans* Fabricius, and waterflea, *Daphnia magna* Straus; and 3) fourteen-day experiments examining formulated reference sediment as a suitable substrate for survival of *H. azteca*, *C. tentans*, *D. magna*, the waterflea, *Ceriodaphnia dubia* Richard, and fathead minnow, *Pimephales promelas* Rafinesque.

To determine responses of commonly used freshwater sediment test species (*H. azteca* and *C. tentans*) to sediment characteristics, formulated and natural freshwater sediments were used to evaluate *H. azteca* and *C. tentans* tolerances of particle size classes and organic matter content of sediments encompassing the range of values encountered in bottom sediments throughout the U.S.

To determine whether copper sulfate is suitable as a reference toxicant for use in whole sediment toxicity tests, *H. azteca* (Saussure) was exposed for 10 days to two field collected sediments amended with copper sulfate. The rationale for selecting copper sulfate as a sediment reference toxicant and proposed selection criteria for sediment reference toxicants was also developed and discussed.

To address concerns regarding the impact of experimental conditions on test results, several experiments were conducted utilizing the organisms *C. tentans*, *H. azteca*, *D. magna*, *C. dubia*, and *P. promelas*. The objectives were to: 1) determine the relative sensitivities of these organisms to copper in water-only exposures and a field collected copper-contaminated sediment; 2) determine the duration of exposure required to elicit mortality or sublethal biological effect (e.g., reduced growth or reproduction) from a copper-contaminated sediment; 3) determine whether or not sublethal test endpoints are more sensitive than survival of test organisms; and 4) illustrate the ability of formulated reference sediment to match selected sediment characteristics and to act as dilution for the copper contaminated sediment, thus providing an accurate means of assessing sediment potency.

This report is divided into four separate sections. Each section represents a separate research task that was part of a more comprehensive research study examining the uncertainty of laboratory sediment toxicity tests in accurately assessing sediment potency. The individual research tasks follow a logical pattern, with results from initial studies influencing subsequent tasks. In Section I, the development and evaluation of formulated reference sediments is presented. Upon successful completion, the results were used to examine benthic organism tolerances of sediment characteristics in Section II, and then used as a reference and dilution sediment in Section III. Results of Section I have been published elsewhere as follows:

Suedel, B.C. and J.H. Rodgers, Jr. 1994. Development of formulated reference sediments for use in freshwater and estuarine sediment tests. *Environmental Toxicology and Chemistry* 13:1163-1175.

Results from Section II have been published elsewhere as follows:

Suedel, B.C. and J.H. Rodgers, Jr. 1994. Responses of *Hyaella azteca* and *Chironomus tentans* to particle-size distribution and organic matter content of formulated and natural freshwater sediments. *Environmental Toxicology and Chemistry* 13:1639-1648.

Material in the current report (Sections I and II) is used with the permission of the Society of Environmental Toxicology and Chemistry (SETAC).

Results from Section III have not been published. Results from Section IV have been published as follows:

Suedel, B.C., E. Deaver and J.H. Rodgers, Jr. 1996. Experimental factors that may affect toxicity of aqueous and sediment-bound copper to freshwater organisms. *Archives of Environmental Contamination and Toxicology*, 30(1): 40-46.

Suedel, B.C., E. Deaver and J.H. Rodgers, Jr. 1996. Formulated sediment as a reference and dilution sediment in definitive toxicity tests. *Archives of Environmental Contamination and Toxicology*, 30(1): 47-52.

Section 1

DEVELOPMENT OF FORMULATED REFERENCE SEDIMENTS FOR FRESHWATER AND ESTUARINE SEDIMENT TESTING

Recently, several concerns have arisen regarding locating and collecting representative, uncontaminated and nontoxic reference and dilution sediments in freshwater and estuarine systems. Frequently, reference sediments possessing sediment characteristics similar to the test sediment are not readily available at nearby localities for use in sediment toxicity tests (Suedel and Rodgers, 1991). In addition, naturally occurring reference sediments may be contaminated, toxic, or contain extant flora or fauna that may inhibit growth or survival of test organisms, thus making the sediment undesirable for use as a reference sediment. A source of representative, uncontaminated and nontoxic sediments is essential for definitive sediment toxicity testing (Suedel and Rodgers, 1995a). Prompted by the requirement for nontoxic reference sediments and unambiguous dilution sediments, formulated reference sediments to be used in a comprehensive sediment toxicity testing or quality evaluation programs were developed. Such sediments have many uses in sediment toxicity testing. These include:

- 1) Answering the question, "How toxic is a sediment?" (i.e., definitive sediment toxicity testing)
- 2) Use as reference and dilution sediments for diverse sediment types in definitive sediment toxicity testing and for use as a control and reference sediment in screening tests addressing the question, "Is a sediment toxic?"
- 3) Determining organism tolerance of sediment characteristics such as particle size distribution, organic carbon content, etc.
- 4) Use as a standard for evaluating the fate and effects of contaminants or specific test compounds in sediments.

"Reference sediment" as used in this document refers to a sediment that is used 1) to dilute a toxic sediment in a definitive sediment test or 2) for evaluating the potency of a test chemical in an amended sediment toxicity test. In these tests, formulated reference sediment would also serve as a control sediment to 1) evaluate unexposed test organism responses and serve as a statistical basis for comparisons of responses of exposed test organisms, and 2) verify that formulated reference sediment is a suitable substrate for test organism survival, growth, or reproduction. Thus, it is crucial that formulated reference sediments yield unambiguous responses by the sediment testing species.

Characteristics of the formulated reference sediment should be amenable to manipulation so the formulated reference sediment can closely approximate important characteristics of the test sediment. Formulated reference sediments were developed and evaluated to match physical and chemical characteristics of field-collected sediments spanning a range of sediment characteristics from throughout the U.S. (Suedel and Rodgers, 1991; Weaver, 1989).

Development and evaluation of formulated reference sediment for use in freshwater and estuarine toxicity testing was initiated at the U.S. EPA Gulf Breeze (ERL) in Gulf Breeze, FL (Walsh *et al.* 1991a,b) where "synthetic" sediments were developed to be used in toxicity tests with wetland plants. "Synthetic" sediment development for use in marine toxicity testing is an active research activity in progress at the Gulf Coast Research Laboratory (GCRL) in Ocean Springs, MS jointly with the U.S. Fish and Wildlife Service (P. Roscigno, personal communication).

The objective of this research was to develop and evaluate formulated reference sediments to be used in definitive sediment toxicity testing. In the following sections, discussion of the rationale behind developing formulated reference sediment is included, and potential formulated sediment constituents and their physical and chemical properties are examined. Formulated reference sediment evaluation includes: 1) matching formulated reference sediment with representative freshwater sediments from the University of Mississippi Biological Field Station (UMBFS) and estuarine sediments from Horn Island, MS; 2) life-cycle experiments examining formulated reference sediment as a suitable substrate for survival and reproduction of the amphipod, *Hyaella azteca* Saussure, midge, *Chironomus*

tentans Fabricius, and waterflea, *Daphnia magna* Straus; and 3) fourteen-day experiments examining formulated reference sediment as a suitable substrate for survival of *H. azteca*, *C. tentans*, *D. magna*, the waterflea, *Ceriodaphnia dubia* Richard, and fathead minnow, *Pimephales promelas* Rafinesque.

MATERIALS AND METHODS

Formulated Reference Sediment Development

Formulated reference sediment constituents were chosen to encompass the range of characteristics of representative freshwater and estuarine bottom sediments occurring throughout the U.S. (Suedel and Rodgers, 1991). Selection criteria for formulated reference sediment constituents included the following:

- 1) Must contain mineral constituents commonly occurring in a variety of soils and sediments throughout the country.
- 2) Must be commercially available or readily collected from a homogeneous (well characterized) deposit in the field.
- 3) Constituents selected should be capable of spanning the range of characteristics encountered in bottom sediments.
- 4) Must be suitable for test organism growth, reproduction and survival in definitive sediment toxicity testing.
- 5) A consistent uncontaminated source must be available.

Clay, silt, sand, and organic constituents were obtained from commercial sources for preparing formulated reference sediments (Table 1-1). Kaolinitic clay, represented by ASP 600® and ASP 900®, is the most ubiquitous aluminosilicate mineral found in soils and bedrock in warm, moist regions of the world, including eastern and southeastern regions of the U.S. (Moore and Reynolds 1989). Kaolinites lack internal surfaces and thus tend to have a relatively low cation exchange capacity (CEC) (1-20 meq/100g) (Weaver, 1989; Grim, 1968). Kaolinite minerals (ASP 400®, ASP 600®, ASP 900®) were obtained from Engelhard Corporation, Pigments and Additives Division, Menlo Park, CA 94025, Edison, NJ, 08818.

Table 1-1. Composition of sands, kaolin based silt and clay, and montmorillonite based clay minerals used to prepare formulated reference sediments (composition according to manufacturers' specifications).

Sands (as oxides)		Kaolin Silt and Clay*		Montmorillonite Clay**	
SiO ₂	97.5%	SiO ₂	45.2%	SiO ₂	60.4%
Fe ₂ O ₃	0.07%	Fe ₂ O ₃	0.3-0.9%	Fe ₂ O ₃	1.4%
Al ₂ O ₃	1.50%	Al ₂ O ₃	38.8%	Al ₂ O ₃	17.6%
Na ₂ O	0.03%	Na ₂ O	0.05-0.3%	P ₂ O ₅	0.02%
TiO ₂	0.04%	TiO ₂	0.6-1.7%	TiO ₂	0.24%
CaO	0.08%	CaO	0.02%	CaO	2.8%
MgO	0.06%	MgO	0.03%	MgO	6.5%
K ₂ O	0.51%	K ₂ O	0.05-0.2%	K ₂ O	0.2%
Other***	0.21%		12.85-14.95%		10.84%

*Components are combined as complex aluminum silicate rather than free oxides.

**From Van Olphen and Fripiat (1979).

***"Other" refers to constituents that were not a part of the mineralogical analyses.

Montmorillonite (smectite) clay from Apache County, Arizona was evaluated and is well characterized. It is one of the "reference clays" chosen by the Clay Minerals Society representing typical clay minerals throughout the U.S. (Van Olphen and Fripiat, 1979; Kerr *et al.*, 1950). Montmorillonite clays are typically derived from volcanic rocks and occur as major constituents in soils of temperate zones (Weaver, 1989). Montmorillonite clays are also abundant in sedimentary rocks and occur in many tropical, subtropical, and arid soils throughout the world (Weaver, 1989). This aluminosilicate clay has a relatively high CEC (120 meq/100g) (Van Olphen and Fripiat, 1979) and can be used to match field-collected sediments with a CEC of >3 meq/100g. Montmorillonite was used in this study because of its presence in soils throughout Mississippi. Montmorillonite clay was obtained from Dr. W.D. Johns, Source Clays, Dept. of Geology, University of Missouri, Columbia, MO, 65211. Although not used in this study, illite is a commonly occurring clay mineral found in sediments (Van Olphen and Fripiat, 1979; Weaver, 1967). If illite predominates in a sediment sample, then illite may be a more appropriate clay constituent than montmorillonite.

The silt fraction of sediments typically consists of very fine sand particles, particulate organic material and agglomerated clay particles. ASP 400® was used to represent the silt fraction of sediments. Although ASP 400® is a kaolinite clay mineral, it is ground to 0.0048 mm diameter and does not separate into clay sized particles upon treatment with dispersant (sodium hexametaphosphate). The silt fraction of sediments refers to particles ranging in size from 0.05 mm to 0.002 mm in diameter (USDA classification system).

Sieved Mystic White® #18 and #90 sands were used to represent coarse (2.0-0.5 mm), medium (0.5-0.25 mm) and fine (0.25-0.05 mm) sand particles of sediments. Mystic White® sands are silica-based minerals that span the range of coarse, medium, and fine sand particles occurring in bottom sediments. Silica sands are found in a variety of freshwater and estuarine sediments where their size distribution is largely controlled by physical processes (Moore 1989). Sands were obtained from New England Silica, Inc., 1370 John Fitch Blvd., South Windsor, CT, 06074.

Organic matter, organic carbon, pH, and CEC are common chemical characteristics that may affect the bioavailability and toxicity of materials in sediments. In this study, humus represented a source of particulate organic matter in formulated reference sediments.

The humus consisted of decaying plant material and manure, which is more representative of naturally occurring organic matter than peat moss. Peat moss has a somewhat restricted distribution and consists of only plant material (Crum, 1988). In some situations where peat moss is present in sediments, it may be a more appropriate source of organic matter than humus. Other sources of humus such as ground plant material typically have a high CEC, ranging from 200 to 400 meq/100g (Bailey and White, 1964), and would be of limited utility due to excessive contribution to CEC content of formulated reference sediment. Humus was obtained from Sims Bark Co. Inc., P.O. Box 896, Tuscumbia, AL, 35674.

Organic matter content of formulated reference sediment was emphasized because organic matter represents a more realistic food source for toxicity testing organisms than organic carbon. Elemental forms of organic carbon are likely not utilized as a food source by benthic organisms. Organic matter is the organic fraction of sediment that includes both fresh and decaying plant, animal, and microbial residues at all stages of decomposition, as well as humus and highly carbonized compounds such as charcoal, graphite, and coal (elemental forms) (Black, 1986). Total organic carbon is contained within the organic matter fraction of sediments and can be estimated as the difference between total carbon and inorganic carbon (Black, 1986).

Dolomite from Wayne County, New York represented a natural bicarbonate buffer occurring in soils and sediments. This source of dolomite is well characterized and is one of the reference minerals chosen by the Clay Minerals Society representing typical minerals occurring in the U.S. (Van Olphen and Fripiat, 1979; Kerr *et al.*, 1950). Dolomite was obtained from Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, New York, 14692. Although not used in this study, calcite is a commonly occurring bicarbonate buffer found in freshwater and marine sediments (Krumbein and Garrels, 1952). Calcite may be a more appropriate bicarbonate buffer than dolomite, depending on location. Formulated reference sediment constituents were characterized with respect to particle size distribution, CEC, solids, organic matter, and pH (Table 1-2).

Table 1-2. Physical and chemical properties of formulated reference sediment constituents after conditioning*.

Constituent	Mineral	Particle Size (µm)	CEC (meq/100g)	Solids (%)	Organic Matter (%)	pH**
ASP 600	clay	0.6	1.59	52.12	13.6	3.5
ASP 900	clay	1.5	2.53	56.21	13.2	3.4
Montmorillonite	clay	<2.0	120	46.59	8.79	7.4
ASP 400	silt	4.8	2.87	62.61	13.2	3.4
Mystic White						
#18 coarse sand	silica	500-850	-***	88.76	1.0	6.8
Mystic White						
#90 medium sand	silica	250-500	-	75.30	0.02	7.0
Mystic White						
#90 fine sand	silica	50-250	-	61.45	1.8	6.8
Humus	organic					
	matter	<2.0 mm	17.3	30.06	86.9	5.9
Dolomite (buffer)	carbonate	<0.05 mm	0.27	84.91	1.31	8.5

*See Table 1-3 for sediment parameters and corresponding analysis.

**pH measurements taken after conditioning each constituent for 7 days.

***Not measured.

Sediment Matching

The proposed formulated reference sediment must be amenable to formulation to match diverse characteristics found in bottom sediments (Suedel and Rodgers, 1991). The objective of the matching phase of formulated reference sediment evaluation was to prepare these sediments to match naturally occurring sediments with respect to particle size distribution, organic matter, CEC, pH, redox potential, and solids (Table 1-3). The accuracy of matching these characteristics was also evaluated. In addition to these parameters, ability to match organic carbon in field-collected sediments was also evaluated (Table 1-3).

The formulated reference sediment constituents, ASP 400® and ASP 600®, were used as representative silt and clay sediment fractions, respectively (Table 1-2). Mystic White® #18 and #90 silica sands were dry sieved into coarse, medium and fine sand fractions on a SoilTest sand shaker. Sand particles correspond to particle size categories following the USDA soil classification system (coarse sand 2.0 - 0.5 mm; medium sand 0.5 - 0.25 mm; fine sand 0.25 - 0.05 mm) (Black, 1986). Dried organic humus was used as a source of organic matter. Humus was dried at 70°C and milled to 2.0 mm in a Wiley Mill before use. Due to the high organic matter content (1.3-13%) of ASP 400® silt, ASP 600® and ASP 900® clays, dolomite, and montmorillonite clay (Table 1-2), these constituents were ashed at 550 °C for 1 hour in a muffle furnace to remove organic matter before use. For buffering capacity, dolomite was added as 1.0% of the total amount of silt required to match a given sediment. In preliminary experiments, it was determined that 1.0% dolomite provided optimal buffering of formulated reference sediments. Four sediments from the UMBFS (Field Sediments 1 - 4) were selected for matching because of their availability in large quantities from pristine areas and because they span the range of sediment characteristics encountered in representative bottom sediments from throughout the U.S. (Suedel and Rodgers, 1991). Two sediments from Horn Island on the Mississippi Gulf Coast, a pristine area with 10-15 ‰ salinity, were used as representative estuarine sediments for matching (Field Sediments 5 - 6).

Formulated reference sediment constituents were mixed dry and hydrated with UMBFS pond water (see Table 1-6 for water chemistry analysis) or synthetic seawater (reconstituted using Instant Ocean per label instructions) prior to analyses. Formulated Reference Sediments 1 - 4 were conditioned for 7 days in cement raceways in flowing (2-3 volume additions per day) UMBFS pond water. Formulated Reference Sediments 5 - 6 were conditioned under static conditions for 7 days. Parameters presented in Table 1-4 were measured after conditioning.

Table 1-3. Sediment Parameters, Corresponding Analysis, and Range of Values Reported for Each Sediment Parameter (from Suedel and Rodgers 1991) except where indicated).

Parameter	Analytical Method	Analysis	Range
Percent Solids	Drying at 104 °C	(a)	11.9-91.7%
Organic Matter	Ashing at 550 °C	(a)	0.3-45% (c,d)
Organic Carbon	High Temp. Induction Furnace (LECO CR-12)	(a)	0.02-11.8%
Cation Exchange Capacity (CEC)	Displacement After Washing	(b)	0.02-71.0 meq/100g
Redox Potential*	Orion Redox Probe	(b)	-409 - +379
pH*	Orion pH Probe	(b)	5.8-9.0 (d,e)
Particle Size Distribution	Hydrometric	(a)	sand = 2-100% silt = 0-95% clay = 0-60%

*Redox and pH measurements were taken at a depth of 1 to 3 cm in all sediment samples.

(a)=Black 1986;(b)=Plumb 1981;(c)=Reynoldson and Hamilton 1982;(d)=Barko and Smart 1986;(e)=Krumbein and Garrels 1952.

Table 1-4. Comparison of field sediments (FS) 1-6 and matching formulated reference sediments (FRS) 1-6 for selected sediment characteristics (mean $\pm$ SD; N=3).

Sediment Number	Sediment Characteristic							
	Solids (%)		Organic Matter (%)		Organic Carbon (%)		CEC (meq/100g)	
	FS	FRS	FS	FRS	FS	FRS	FS	FRS
1	72.95	79.13	1.7	1.1	0.38	0.37	1.50	0.74
	± 1.24	± 0.21	± 0.64	± 0.04	± 0.005	± 0.04	± 0.25	± 0.09
2	59.98	59.23	6.8	6.6	1.4	3.0	4.18	4.17
	± 5.76	± 0.25	± 0.25	± 0.26	± 0.04	± 0.05	± 0.25	± 0.10
3	76.35	77.45	0.30	0.17	0.021	0.014	0.19	0.08
	± 0.28	± 0.77	± 0.087	± 0.089	± 0.020	± 0.015	± 0.044	± 0.01
4	41.51	58.85	7.8	8.4	2.21	2.76	7.8	3.1
	± 0.29	± 0.06	± 0.16	± 0.19	± 0.03	± 0.05	± 2.61	± 0.38
5	78.62	77.74	0.33	0.27	0.05	0.02	0.12	0.11
	± 0.75	± 0.24	± 0.010	± 0.026	± 0.002	± 0.011	± 0.03	± 0.03
6	75.73	66.25	7.64	6.18	1.82	2.73	5.1	2.2
	± 0.45	± 12.1	± 0.35	± 0.17	± 0.27	± 0.03	± 1.10	± 0.06
Mean								
Difference($\pm$ SE)	2.26 $\pm$ 3.65		0.32 $\pm$ 0.28		0.478 $\pm$ 0.26		1.41 $\pm$ 0.80	



Table 1-4 (Cont'd). Comparison of field sediments (FS) 1-6 and matching formulated reference sediments (FRS) 1-6 for selected sediment characteristics (mean $\pm$ SD; N=3).

Sediment Number	Sediment Characteristic							
	Redox (mv)		pH		Coarse Sand (%)		Medium Sand (%)	
	FS	FRS	FS	FRS	FS	FRS	FS	FRS
1	+57	+238	6.0	6.9	8.4	9.2	31.7	23.0
	± 21	± 9	± 0.06	± 0.06	± 3.41	± 0.85	± 1.13	± 0.16
2	-168	+217	6.9	6.5	2.5	2.9	5.5	6.0
	± 6	± 4	± 0.06	± 0.06	± 1.59	± 0.12	± 0.64	± 0.10
3	+252	+235	6.5	7.5	11.3	6.5	61.9	36.0
	± 16	± 3	± 0.12	± 0.06	± 1.79	± 0.29	± 0.70	± 0.98
4	-140	+231	6.5	6.8	0.2	0.6	1.0	1.2
	± 17	± 1	± 0.15	± 0.06	± 0.06	± 0.03	± 0.15	± 0.03
5	-100	+223	6.6	7.5	9.7	6.5	64.0	36.0
	± 25	± 4	± 0.06	± 0.06	± 1.65	± 0.29	± 2.90	± 0.98
6	-110	+239	6.9	6.3	0.8	1.9	8.1	11.0
	± 9	± 6	± 0.17	± 0.10	± 0.84	± 0.09	± 1.89	± 0.07
Mean								
Difference($\pm$ SE)	267 $\pm$ 64*		0.37 $\pm$ 0.30		0.88 $\pm$ 1.01		9.86 $\pm$ 5.66	



Table 1-4 (Cont'd). Comparison of field sediments (FS) 1-6 and matching formulated reference sediments (FRS) 1-6 for selected sediment characteristics (mean $\pm$ SD; N=3).

		Sediment Characteristic							
		Fine Sand (%)		Total Sand (%)		Silt (%)		Clay (%)	
Sediment Number		FS	FRS	FS	FRS	FS	FRS	FS	FRS
1-12	1	35.8	40.7	75.9	72.8	21.4	27.2	2.7	0.0
		± 4.56	± 0.97	± 1.79	± 0.55	± 4.10	± 0.55	± 2.31	± 0.0
	2	8.8	13.1	16.8	22.0	82.6	78.0	1.0	0.0
		± 1.42	± 0.20	± 3.25	± 0.25	± 4.51	± 0.42	± 0.89	± 0.0
	3	19.7	46.7	93.1	92.0	6.9	8.0	0.0	0.0
		± 1.13	± 1.15	± 0.51	± 2.21	± 0.51	± 2.27	± 0.0	± 0.0
	4	2.5	5.3	3.8	7.1	93.8	92.9	2.4	0.0
		± 0.21	± 0.03	± 0.36	± 0.75	± 3.79	± 0.75	± 4.10	± 0.0
	5	19.8	46.7	93.5	89.2	6.5	10.8	0.0	0.0
		± 4.49	± 1.15	± 1.19	± 0.55	± 1.19	± 0.55	± 0.0	± 0.0
	6	65.3	56.5	74.2	69.4	20.2	27.2	5.6	3.5
		± 2.75	± 0.00	± 1.00	± 0.23	± 1.20	± 0.95	± 0.20	± 0.98
Mean									
Difference($\pm$ SE)		9.5 $\pm$ 5.87		0.83 $\pm$ 1.71		0.76 $\pm$ 1.88		1.36 $\pm$ 0.49*	

*Indicates significant difference at the 0.05 level.

Organism Tolerance of Formulated Reference Sediment

The suitability of formulated reference sediment as a reference or control sediment depends on its ability to provide test organisms sufficient habitat so these organisms can survive and reproduce with no adverse effects. In addition, "normal" test organism behavior such as burrowing, case building, grazing, etc. should occur. Experiments for determining organism performance in formulated reference sediment should include experiments spanning test organisms' entire life-cycles. These experiments were performed by culturing organisms in formulated reference sediment, which is more rigorous than conducting experiments of 10-14 days duration where only a fraction of an organism's life history is taken into account. *D. magna* (water column), *H. azteca* (epibenthic) and *C. tentans* (infaunal) were selected for experimentation because they occupy different niches in freshwater aquatic habitats and are sensitive to a wide variety of toxic materials occurring in aquatic systems. These organisms were cultured using formulated reference sediment and Field Sediment 1 (control) as substrates to determine the effect of these sediments on organism survival or reproduction. Field Sediment 1 was selected as a control sediment because it was used successfully in previous sediment toxicity testing programs with the organisms used in this study (Suedel and Rodgers 1994a). Previous organic and inorganic analyses of Field Sediment 1 indicated below detection limits for organics and near detection limits for metals except iron and aluminum (associated with smectite clays).

When hydrated without conditioning, formulated reference sediment constituents were not suitable for *H. azteca* survival in preliminary experiments (see Table 1-5 and related discussion). Therefore, a conditioning period was required before conducting additional experiments. Conditioning involved placing each constituent (ASP 400®, ASP 600®, ASP 900®, sands, humus) separately in plastic tubs (60 x 40 x 22 cm) and hydrating with UMBFS pond water. Tubs containing hydrated constituents were placed in flow-through (2-3 volume additions per day) cement raceways at the UMBFS until colonization by various invertebrate species was noted on the sediments. The presence of various oligochaetes, midges, isopods and algae was noted on the surfaces of constituents after 7 days of conditioning. The presence of indigenous invertebrate species on these constituents initially signaled the end of the conditioning period for test organisms and for formulations of reference sediments.

Table 1-5. Exposure of *H. azteca* to unconditioned formulated reference sediment constituents for 10 days.

Constituent	Number Surviving/Total	Percent Survival
100% coarse sand	2/10	20%
60% coarse sand, 40% medium sand	3/10	30%
60% medium sand, 40% ASP 400 silt	0/10	0%
60% medium sand, 40% ASP 600 clay	0/10	0%
40% medium sand, 60% ASP 600 clay	0/10	0%

After the conditioning period and before mixing, invertebrates on the sediment surface and standing water were removed. The sediment constituents were returned to the laboratory and stored wet at room temperature (22 ± 2 °C) up to 30 days before testing.

In initial non-replicated *D. magna*, *H. azteca*, and *C. tentans* culture experiments, aquaria were maintained under ambient laboratory conditions for temperature (25 ± 1 °C) and light (16 h light/8 h dark photoperiod). For each organism, the experiment was conducted in two 39 L glass aquaria containing either 2.0 kg of Field Sediment 1 (as a control) or 2.0 kg of formulated reference sediment, with approximately 15 L of filtered UMBFS pond water

overlying each sediment. In these experiments, formulated reference sediment was prepared to match Field Sediment 1 with respect to particle size distribution (i.e., 8% coarse sand, 32% medium sand, 36% fine sand, 21% silt, and 3% clay). Humus was also added, achieving a concentration of 0.1% organic matter. All formulated reference sediment constituents were reference sediment (Table 1-2) and were previously conditioned for 7 days. ASP 600® and ASP 900® each represented 50% of the clay fraction on a dry weight basis. After adding 30 L of filtered UMBFS pond water to both sediment treatments, contents of the culture aquaria were allowed to equilibrate and suspended particles to settle for 24 hours before introducing test organisms.

The experiment with *D. magna* was initiated by adding 50, <48 hours old neonates to each aquarium with a 6 mm ID glass pipet and maintained for 28 days. This exposure was sufficient for exposure of juvenile, adolescent, and adult life stages producing several broods of *D. magna* neonates. Only the egg stage was not exposed to formulated reference sediment. *D. magna* were fed 5 ml (approximately 5×10^6 cells/ml) of *Selenastrum capricornutum* every other day. The aquaria were not aerated. After 28 days, *D. magna* in each aquarium were collected and counted.

The experiment with *C. tentans* was initiated by adding two egg masses to each aquarium. Each egg mass consisted of approximately 200 to 500 eggs. Aquaria were vigorously aerated and *C. tentans* were fed 2-3 ml of a Tetra conditioning food (Tetrawerke, Germany) suspension daily. This feeding rate has been used to successfully culture *C. tentans* at our laboratory for 2 years (see below). *C. tentans* were cultured until all larvae had pupated and emerged as adult midges (60 days). Although not technically a full life-cycle exposure, larval, pupae, and adult life stages were exposed. Only the *C. tentans* eggs were not exposed.

The *H. azteca* experiment was initiated by adding 100 adults (2 - 3 cm length) to each aquarium. *H. azteca* were fed ground maple leaves (*Acer rubrum*) once per week as needed. Both aquaria were gently aerated (2 bubbles/sec). *H. azteca* were cultured for 2 months, sufficient time for one complete life cycle at room temperature (24-25 °C) (de March 1981). At the end of two months, *H. azteca* in each aquarium were collected and counted.

For a more rigorous evaluation of formulated reference sediment, replicated experiments were also conducted with *D. magna* and *C. tentans*. The experiment with *D. magna* (28 days duration) was initiated by placing two <24 hour-old *D. magna* neonates per 250 ml beaker (N=5 replicates/sediment) containing 160 ml of alkalinity and hardness adjusted (see culture procedures) UMBFS pond water (alkalinity and hardness of unadjusted UMBFS pond water is outside the tolerance range of *D. magna*) and either 40 ml of Field Sediment 1 (control) or formulated reference sediment. *D. magna* were fed *S. capricornutum* daily. The overlying water from each beaker was partially renewed (80-100 ml) weekly by gently pouring water on a watch glass to minimize disturbance. Neonates were removed from each beaker and counted every 2-3 days. The water was not aerated since dissolved oxygen concentrations did not fall below 40% saturation.

The replicated experiment with *C. tentans* (40 days duration) was initiated by placing from 5-8 <24 hour-old first instar *C. tentans* larvae to each 250 ml beaker (N=5 replicates/sediment) containing 160 ml UMBFS pond water and either 40 ml of Field Sediment 1 or formulated reference sediment. *C. tentans* were fed 0.05 ml of a cerophyll suspension every other day, and 80-100 ml of the overlying water was renewed weekly. *C. tentans* survival, growth, and development (qualitative) was monitored throughout the experiment. The water was not aerated since dissolved oxygen concentrations did not fall below 40% saturation.

Formulated reference sediment used in replicated culture experiments contained all of the constituents listed in Table 1-2 and was prepared as follows: percent solids - 64.0%; organic matter - 7.6%; redox potential - +228 mv; CEC - 3.61 meq/100g; pH - 6.2; coarse sand - 3.1%; medium sand - 5.1%; fine sand - 14.6%; silt - 77.2%; clay - 0.0%. This formulated sediment was prepared separately and was not designed to match field collected sediments listed in Table 1-4.

In addition to conducting chronic experiments, static 14 day whole sediment exposures were conducted with *C. tentans* (10 days old, 2nd instar), *H. azteca* (2-3 weeks old), *D. magna* (24-48 h old), *C. dubia* (<24 h old), and *P. promelas* (3-4 d old) using formulated reference sediments. These experiments (except for *C. dubia*) were conducted in 250 ml

beakers with 40 ml formulated reference sediment and 160 ml UMBFS pond water. There were 4 replicate beakers for each organism, with 8 organisms per beaker (6 for *C. tentans*). The *C. dubia* experiment was conducted in 50 ml beakers with 8 ml formulated reference sediment and 32 ml UMBFS pond water. *C. dubia* experiments were initiated with 10 replicate beakers, each containing one <24 hour-old neonate. Characteristics of formulated reference sediment used in 14 d whole sediment exposures were the same as those used in replicated exposures (see previous paragraph). Test organisms were fed as described previously. *C. dubia* were fed 0.5 ml (approximately 5×10^6 cells/ml) *S. capricornutum* daily and *P. promelas* were fed newly hatched *Artemia* nauplii daily.

Sediment Test Organism Culture Procedures

All test organisms were cultured at the UMBFS. *D. magna* and *C. dubia* culturing procedures followed the methods of U.S. EPA (U.S. EPA 1985a, 1989). *D. magna* and *C. dubia* cultures were maintained in UMBFS pond water in light (16 h light/8 h dark photoperiod) and temperature (21 ± 1 °C) controlled incubators. UMBFS pond water is characteristically soft, with low levels of dissolved ions. Hardness and alkalinity were adjusted with (0.1 g/L) NaHCO_3 and CaCl_2 to a total hardness of 80 mg/L as CaCO_3 and an alkalinity of 60 mg/L as CaCO_3 . *D. magna* and *C. dubia* cultures were fed *S. capricornutum* daily.

H. azteca and *C. tentans* cultures were maintained under ambient temperature conditions (22 ± 2 °C) in 39 L glass aquaria containing filtered, dechlorinated tap water. *H. azteca* culturing procedures followed the methods of de March (1981). *H. azteca* were cultured using maple leaves (*Acer rubrum*) as a substrate and were fed ground rabbit chow pellets (Clover Brand, Madison, MS) twice each week. Amphipods used for testing were gently poured through a series of stainless steel sieves. Those individuals passing through a 1000 um mesh sieve but retained by a 600 um mesh sieve were used for testing. *C. tentans* culture methods followed those of Townsend *et al.* (1981). *C. tentans* culture substrate consisted of shredded brown paper towels (#ML96 Georgia Pacific, Battleboro, VT) to a depth of three cm. A suspension (0.1 g food/1 ml water) of Tetra conditioning food

(Tetrawerke, Germany) and a cerophyll (Ward's Nat. Sci., Rochester, NY) suspension (0.025 g cerophyll/1 ml water) was introduced to the cultures daily.

P. promelas culture methods followed those of U.S. EPA (1985a). *P. promelas* cultures were maintained under ambient temperature (25 ± 2 °C) and light (16 h light/8 h dark photoperiod) conditions. Fish were cultured in 60 L glass aquaria under flow through conditions with filtered, dechlorinated tap water. Fish were fed a daily diet consisting of Tetra staple food (Tetrawerke, Germany) and frozen brine shrimp (San Francisco Bay Brand, Newark, CA).

Statistical Analysis

Mean differences of formulated reference sediment characteristics were statistically compared with those of natural sediments using paired t-tests (Zar 1984; Sokal and Rohlf 1981). The mean difference within each pair (field sediment vs. formulated reference sediment) for each sediment characteristic was compared for all six sediments matched (N=6; i.e., the number of pairs of data). Respectively, univariate and t-test procedures were used to determine normality and differences in means from replicated life-cycle experiments with *C. tentans* and *D. magna* cultured on UMBFS sediment 1 or formulated reference sediment (SAS 1989). Analysis of variance and Tukey's test (SAS 1989) were used to determine differences between means of sediment organic matter required for *C. tentans* survival in 10 day tests. The 5% alpha level was used in all statistical tests.

RESULTS AND DISCUSSION

Formulated Reference Sediment Development

Formulated reference sediment constituents spanned the range of particle sizes encountered from fine clay (0.6 μ m) to coarse sand (850 μ m) [Table 1-3]. Cation exchange capacity (CEC) for clay and silt constituents ranged from 1.6 to 2.9 meq/100g, which would encompass only 50% of the CEC values encountered in freshwater and marine sediments from throughout the U.S. (Table 1-3). Sediments with a CEC of >2.9 meq/100g require an additional clay constituent such as montmorillonite to match sediments with CECs >2.9 meq/100g. Solids concentrations varied from near 46% for montmorillonite clay to near 90%

for coarse sand, encompassing the range of solids concentrations found in bottom sediments (Table 1-3). Organic matter content of formulated reference sediment constituents ranged from 0.02% for medium sand to 89.6% for humus. Organic matter content of silt and clay constituents ranged from 8.8% to 13.6%, and these constituents may require ashing at 550 °C to remove organic matter to permit matching sediments with organic matter <10%. Addition of humus (organic matter content = 86.9%) to formulated reference sediment will allow matching virtually all concentrations of organic carbon and organic matter that may be encountered in field collected sediments.

Although sediments were matched quantitatively with respect to organic matter, qualitative differences between sediments may still exist, due to the various forms of organic carbon present in sediments (e.g. mineral forms such as coal, dissolved and particulate forms). The humus used in this study was not characterized further than shown in Table 1-2. Complete characterization between batches of organic matter and organic carbon sources is essential to monitor the effects of source, degree of decomposition, and potential for contamination. Sources of organic matter and organic carbon are not typically exposed to rigorous quality assurance and quality control procedures as are the other constituents used (sands, clays), as sand and clay constituents are used in various products designated for human use.

As illustrated above, mineral constituents of formulated reference sediments are commonly found in soils and sediments throughout the U.S. and are commercially available. Preparation of sand, silt and clay constituents in appropriate quantities should permit formulation of sediments that span the range of sediment characteristics encountered in practice (Table 1-3). Because the quantities of sand, silt, clay, and humus are supplied in substantial amounts (19-37 kg), several experiments or studies can be conducted with a single batch, minimizing the potential for inconsistencies between batches. In addition, many of these constituents (clay, silt, dolomite) are considered reference materials or are used extensively for human use and thus are subject to rigorous Quality Assurance/Quality Control procedures to ensure consistency between batches. Sources of organic matter or organic carbon (e.g., potting soil, humus) are typically not subjected to rigorous QA/QC procedures, as they are not used in applications subject to strict requirements. Further evidence

demonstrating formulated reference sediments' ability to encompass sediment characteristics and suitability of these constituents for organism survival, growth and reproduction are discussed in the following sections.

Sediment Matching

Table 1-4 presents the results of an effort to match formulated reference sediments with freshwater sediments (Field Sediments 1-4) and estuarine sediments (Field Sediments 5 and 6). Sediment solids values of formulated reference sediments were similar to all field sediments. Solids concentrations of formulated reference sediments were within 6% of all field sediments except for a 17% difference (41.5% vs. 58.9%) between Field Sediment 4 and Formulated Reference Sediment 4 (Table 1-4). These data indicate that formulated reference sediment can match a wide range of solids concentrations, with the exception of field collected sediments with silt composition >90% (e.g., Field Sediment 4). Solids concentration of sediments is negatively correlated with silt content of sediments, i.e., as silt content increases, solids concentration decreases (Suedel and Rodgers, 1991). Although sediments with >90% silt content are relatively uncommon (Suedel and Rodgers, 1991), these sediments may be difficult to match with formulated reference sediment with respect to solids concentration. Differences between the composition of silt fractions of field collected and formulated reference sediment may significantly affect solids concentration when present in sufficient quantities (e.g. >90%).

Organic matter content of formulated reference sediments was not significantly different from field collected sediments studied (Table 1-4). Organic matter content of formulated reference sediment was within a factor of two for all field sediments, indicating that field sediments with varying organic matter content can be matched by formulated reference sediments.

Organic carbon values of formulated reference sediments were not statistically different from field sediments characterized, even though no attempt was made to match sediments with respect to organic carbon. Except for Field Sediments 2 and 5, organic carbon values varied by a factor of two or less. Matching for organic carbon was less successful than organic matter because the organic carbon fraction of organic matter is not

quantitatively consistent between sediments. From field sediments used in this study, the organic carbon fraction of organic matter ranged from 10 to 50%. Therefore, matching for organic matter will not necessarily result in matching organic carbon. Nevertheless, these data indicate that sediments can be matched for organic carbon content.

CEC values of formulated reference sediments were not statistically different from CEC values of field sediments (Table 1-4). All CEC values of formulated reference sediments were within a factor of 2.5 of field collected sediments. CEC is dependent upon a number of factors such as pH and cations present in water (Black, 1986), thus the formulated reference sediment CEC data in this study reflect UMBFS pond water characteristics used to hydrate these sediments. CEC values obtained for formulated reference sediment will be influenced in part by the source water used to condition formulated reference sediment as well as the overlying water used in whole sediment toxicity tests.

Redox potentials for all formulated reference sediments were oxidized, with measured Eh values between +210 to +240 mv, considerably higher than Eh values measured for field collected sediments (except Field Sediment 3). Formulated reference sediment as prepared herein did not match sediments exhibiting slightly reduced (+100 to -100 mv) or reduced conditions (less than -100 mv) without further manipulation. Matching redox potential of sediments may be important in situations where formulated reference sediment is used for diluting metal-contaminated sediments or when amending formulated reference sediment with metals. Redox has been hypothesized to influence metal bioavailability (Gambrell and Patrick, 1988). Redox potential of sediments has been controlled in previous studies by supplying appropriate quantities of oxygen, nitrogen, or helium gas in an incubated system (Hambrick *et al.* 1980).

Formulated reference sediment pH values were similar to field collected sediments (Table 1-4). Formulated reference sediment pH values were within one pH unit of all field collected sediments, indicating some success in matching pH values. As noted in Table 1-3, pH values for clay, silt and humus constituents of formulated reference sediment were below 6.0. The buffering capacity of dolomite added to each sediment was sufficient to increase the pH of each formulated reference sediment to near neutral values (6.3 to 7.5). However, a period of up to 7 days was required to achieve stable pH readings in formulated reference sediments (Figure 1-1).

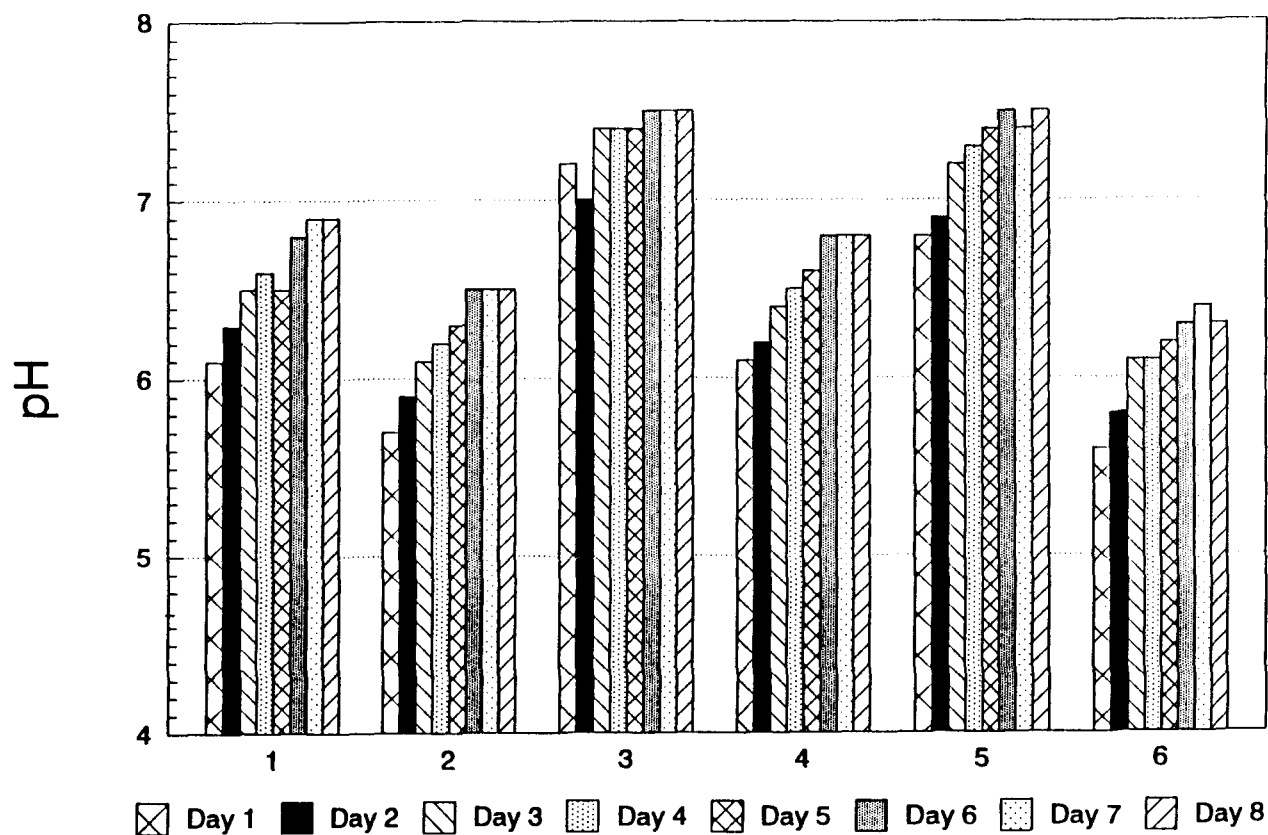
There was considerable success in matching particle sizes of field collected sediments, as significant differences were not observed for coarse, medium, fine, total sand, and silt (Table 1-4). However, clay content of formulated reference sediments was significantly different from field sediments. This reflected the lack of variability (0.0% in many cases) between clay samples. Coarse and total sand particles of formulated reference sediment matched within 5% of all field collected sediments examined. However, medium and fine sand fractions of Formulated Reference Sediments 3 and 5 were considerably different than Field Sediments 3 and 5. These differences between medium and fine sand particles were likely due to the use of different sieves, one sieve series for preliminary separation of sand particles (SoilTest shaker), and another sieve series used for particle size determinations. This underscores the importance of using identical sieves for all particle size determinations and constituent preparation.

Formulated reference sediments can be prepared to match naturally occurring sediments with respect to particle size distribution, solids, OC, CEC, pH, and organic matter content. It is recommended that silt, clay and dolomite constituents be ashed before use to remove excess dissolved organic matter, especially when matching sediments with low organic matter content. It is not assumed that factors influencing sorption and bioavailability remain unaltered, although the suitability of test organism survival, growth, and reproduction of formulated reference sediments containing ashed constituents may remain unaltered. This would need to be verified on a case by case basis.

Organism Tolerance of Formulated Reference Sediment

Conditioning Formulated Reference Sediments

When hydrated without conditioning, formulated reference sediment constituents were not suitable for *H. azteca* survival in preliminary experiments (Table 1-5) and therefore a conditioning period prior to conducting experiments was initiated. The conditioning period was designed to add an aquatic biological component to formulated reference sediment which was initially absent in unconditioned formulated reference sediment. Addition of microbial species (e.g., bacteria, algae) to formulated reference sediment may provide test organisms a



Formulated Reference Sediment

Figure 1-1. Time (days after initial mixing) required for stabilization of pH values of formulated reference sediments prepared to match field collected sediments in this study.

more suitable substrate in which to survive and reproduce, and may also serve as a food source as well. Conditioning formulated reference sediment with UMBFS pond water resulted in a biological component in formulated reference sediment that is unique to the UMBFS and will vary, depending on the source of the water used to condition formulated reference sediment. Although colonization of formulated reference sediment by indigenous oligochaete and midge species occurred in this study, in practice, formulated reference sediment should be conditioned in a natural water source (i.e., flow-through, static, or static-renewal) that includes various microbial species but not indigenous macroinvertebrate species such as oligochaetes and midges, which can interfere with test organism performance. Toxicity observed with unhydrated formulated reference sediment should not be viewed as unusual since experiments conducted with dried (70 °C) Field Sediment 1 also resulted in toxicity (>50%) to *H. azteca* and *C. tentans*.

D. magna Experiments

In the non-replicated experiment exposing *D. magna* to Field Sediment 1, 100% mortality was observed within 14 days (Table 1-6). Although eggs were observed in the brood chamber of some adults, no release of live offspring from these adults was observed. This was not surprising on account of the low levels of dissolved salts present in the water and the fact that *D. magna* can not be successfully cultured in unaltered UMBFS pond water. Naturally occurring populations of *D. magna* are generally restricted to waters exceeding hardness values of UMBFS pond water (6-24 mg/L as CaCO₃; U.S. EPA, 1985a). *D. magna* cultured on formulated reference sediment, however, demonstrated rapid growth and reproduction during the exposure period resulting in 2234 *D. magna* present in the culture after 28 days (Table 1-6). Characteristics of UMBFS pond water were considerably altered when overlying formulated reference sediment and thus were different than pond water overlying Field Sediment 1. Increased pH, conductivity, alkalinity and hardness values resulting from placement of UMBFS pond water on this sediment likely contributed to the successful culture of *D. magna* on formulated reference sediment. *D. magna* were also observed vigorously foraging on formulated reference sediment at the sediment-water interface.

These results illustrate that sediments can affect the properties of water placed over sediment in sediment toxicity testing. Water placed on a sediment will reflect the general physical and chemical characteristics of that sediment, regardless of the water source. UMBFS pond water contains low levels of dissolved salts when overlying UMBFS sediments, but when UMBFS pond water is placed on another sediment (e.g., formulated reference sediment), it is altered by the physical and chemical properties of that sediment. Other sources of water placed on formulated reference sediment will likely be altered by formulated sediment, regardless of the water source because water removed from its original sediment and placed on formulated reference sediment will change with respect to chemical properties (e.g. pH, hardness, alkalinity, conductivity). Likewise, reconstituted water placed on bottom sediments will be chemically altered by the sediment and altered differently by dissimilar sediments. A viable area of future research could be to investigate the variability and degree of influence of overlying water on sediments and vice versa.

The results from the replicated 28 day experiment with *D. magna* showed 100% survival when exposed to both sediments with adjusted UMBFS pond water (Table 1-6). The mean number of offspring per replicate ($\pm$ S.D.) was 95.2 ± 5.26 and 91.5 ± 7.33 for Field Sediment 1 (control) and formulated reference sediment, respectively, and were not significantly different from each other. These data provide additional evidence that formulated reference sediment can provide suitable habitat for *D. magna* through 28 d of exposure.

C. tentans Experiments

The results from the unreplicated experiment exposing *C. tentans* to Field sediment 1 showed no larvae achieving the adult life-stage after 60 days (Table 1-7). The presence of numerous other indigenous organisms such as oligochaetes and other species of midges observed in Field Sediment 1 may have contributed to the mortality of *C. tentans*. Other species of midges and oligochaetes were observed in abundance and likely preyed upon or outcompeted *C. tentans* for food and space. No third or fourth instar *C. tentans* larvae or

Table 1-6. Results of water chemistry analysis and reproductive success of *D. magna* cultured on Field Sediment 1 and formulated reference sediment for 28 days.

Parameter	Non-replicated Experiment***		Replicated Experiment***	
	Field Sediment 1	Formulated Sediment	Field Sediment 1	Formulated Sediment
Temperature	25-26°C	25-26°C	20.1-22.6°C	20.0-22.6°C
Dissolved Oxygen (mg/L)	7.2-8.0	8.0-9.8	7.5-8.4	7.4-8.6
pH	6.2-6.5	7.6-7.9	7.1-7.7	7.8-7.9
Conductivity (umhos/cm)	40-60	140-155	230-290	320-350
Alkalinity (mg/L as CaCO ₃)	5	48	12-28	56-68
Hardness (mg/L as CaCO ₃)	12	56	8-12	12-16
<i>D. magna</i> survival	0*	2234*	(10/10)=100%	(8/8)=100%
<i>D. magna</i> reproduction			95.2±5.26**	91.5±7.33**

*Total number of *D. magna* present at test termination (initial N=50).

**Mean (±S.D.) number of neonates produced per replicate during the 28 d exposure.

***Unaltered UMBFS pond water was used for the non-replicated experiment and altered UMBFS pond water was used in the replicated experiment.