

# Summary of a SETAC Technical Workshop

## Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations with a Review of Methods and Applications, and Recommendations for Future Areas of Research

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Summary of the SETAC Workshop on “Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations with a Review of Methods and Applications, and Recommendations for Future Areas of Research,” held 18–22 March 2000, in Pensacola, Florida, USA.

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Published May 2001.

Publication sponsored by the Society of Environmental Toxicology and Chemistry (SETAC) and the SETAC Foundation for Environmental Education.

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Reference Listing: Carr RS, Nipper M, Adams WJ, Berry WJ, Burton Jr GA, Ho K, MacDonald D, Scroggins R, Winger PV. 2001. Summary of a SETAC technical workshop: Porewater toxicity testing: Biological, chemical, and ecological considerations with a review of methods and applications, and recommendations for future areas of research. Summary of the SETAC Workshop on Porewater Toxicity Testing: Biological, Chemical, and Ecological Considerations with a Review of Methods and Applications, and Recommendations for Future Areas of Research; 18–22 March 2000; Pensacola, FL. Society of Environmental Toxicology and Chemistry (SETAC). Pensacola, FL. 38 p.

This summary outlines results of the workshop entitled “Porewater toxicity testing: Biological, chemical, and ecological considerations with a review of methods and applications, and recommendations for future areas of research,” sponsored by SETAC on 18–22 March, 2000, in Pensacola, Florida, USA. The workshop included 30 participants from 7 countries, including the U.S., Canada, Mexico, United Kingdom, Italy, Australia, and New Zealand, who came together over 5 days to examine the current uses of porewater toxicity tests in sediment quality assessment and testing. The results are presented here in summary, including the recommendations of 5 workgroups that show how interconnected the issues involved are. A more in-depth report of the proceedings, including the recommendations presented here, the discussions from each workgroup, the research needs identified during the workshop, and a series of review chapters on the key issues involved, is being prepared for publication through SETAC.

*Keywords: pore water, toxicity testing, sediment quality triad (SQT), toxicity identification evaluation (TIE)*

## Scientific Acronyms Used in Text

AET:	Apparent effects threshold	ESG	Equilibrium partitioning sediment guidelines
AVS:	Acid volatile sulfide	HOC:	Hydrophobic organic compound
BOD:	Biological oxygen demand	PEL:	Probable effects level
COCs:	Chemicals of Concern	SEM:	Simultaneously extractable metals
COD:	Chemical oxygen demand	SQG:	Sediment quality guidelines
DO:	Dissolved oxygen	SQT:	Sediment quality triad
DOC:	Dissolved organic carbon	TEL:	Threshold effects level
ERL:	Effects range – low	TIE:	Toxicity identification evaluation
ERM:	Effects range – median	TOC:	Total organic carbon
EqP:	Equilibrium partitioning	TSS:	Total suspended solids
		WWTP:	Wastewater treatment plant

## Introduction

Aquatic toxicology has developed significantly since the initial recognition that toxicity tests represent useful and reliable tools for evaluating the adverse effects of chemicals discharged into water bodies. In the 1970s, environmental scientists realized that sediments act as a sink for contaminants and, as a result of this finding, the development of sediment toxicity testing had its beginning (Swartz et al. 1979; Gannon and Beeton 1971). Sediment pore water was soon identified as a major route of exposure of infaunal and epibenthic organisms to sediment contaminants (USEPA 1993; Di Toro et al. 1991; Adams et al. 1985). Several methods have been developed for the extraction (Carr and Chapman 1995; Winger and Lasier 1991; Jahnke 1988; Hesslin 1976; Edmunds and Bath 1976; Presley et al. 1967) and toxicity testing of pore water with aquatic organisms (Carr 1998; Hooten and Carr 1998; Ankley et al. 1992; Carr et al. 1989). In addition, the sensitivity of porewater toxicity test methods has been compared with that of solid-phase tests (Carr et al. 2000; Nipper et al. 1998; Carr, Chapman, Howard et al. 1996; Carr, Long et al. 1996; Sarda and Burton 1995; Carr and Chapman 1992).

As a result of porewater research developments, a number of critical issues have been identified regarding porewater extraction and toxicity testing methods. For example, questions have been raised regarding the effect of sediment sampling and handling procedures and of porewater extraction methods on the bioavailability of contaminants. In addition, it has been suggested that the influence of confounding factors (e.g., ammonia, sulfides, dissolved oxygen [DO], pH, salinity) on different test methods and on the interpretation of porewater tests results needs to be evaluated. Guidance on the use of pore water for sediment Toxicity Identification Evaluations (TIE) is also needed. Furthermore, the reliability of porewater test data for assessing potential adverse effects of contaminants to the benthic biota and their use in the weight of evidence type assessment (e.g., sediment quality triad (SQT) studies) need to be established. Finally, the correlation of porewater test results with sediment quality guidelines (SQGs) and the potential uses of porewater tests in various regulatory processes need to be elucidated.

The applicability of porewater tests for sediment quality assessments is of critical importance for a number of environmental assessment and monitoring programs throughout the U.S. and elsewhere. While intensive research has been conducted on various issues related to porewater testing in recent years, several issues associated with these procedures and their applications have yet to be fully resolved. For this reason, the Society of Environmental Toxicology and Chemistry (SETAC) and its partners (see list at the end of this document) convened a technical workshop to review the existing methods and applications associated with porewater sampling and toxicity testing and to develop a series of recommendations related to the use of these tools. The workshop was also designed to facilitate the identification of future research needed to resolve any outstanding uncertainties related to these topics.

## Workshop Purpose and Goals

A Technical Workshop promoted by SETAC was convened to facilitate discussions among a panel of experts on porewater toxicity testing and its uses with the objective of providing both environmental regulators and the scientific community with state-of-the-science guidance on the applications of porewater test procedures. The specific objectives of the workshop were

to discuss important aspects of porewater toxicity testing and, whenever possible, to develop consensus recommendations, based on the best available knowledge, on the most appropriate and reliable uses and applications of porewater toxicity tests.

This summary outlines the results and conclusions of the SETAC Technical Workshop that was held in Pensacola, Florida, USA, on 18–22 March 2000, which critically examined the current uses of porewater toxicity tests in sediment quality assessment and monitoring. The complete workshop proceedings document is currently in preparation for publication in book form by SETAC. The proceedings will present a summary of the discussions, recommendations, and research needs identified by each workgroup, as well as a series of review chapters that address key issues related to porewater sampling and toxicity testing and the use of these tools in sediment quality assessment studies and the regulatory arena.

## Participation and Format

The workshop followed the format of previous SETAC Pellston and Technical Workshops. As such, a wide array of specialists in different aspects of porewater testing research and applications were invited to participate in the workshop. In total, 30 participants from 7 countries (U.S., Canada, Mexico, United Kingdom, Italy, Australia, and New Zealand) assembled in Pensacola, Florida, to engage in 4 days of intensive discussions. These participants, selected based on their expertise and affiliation, included chemists, toxicologists, benthic ecologists, and regulators, representing universities, government research, regulatory agencies, mining and chemical industries, consulting agencies, as well as a non-government organization (NGO).

The workshop began with a series of plenary talks and a guest speaker. These presentations provided reviews of several porewater-related issues and identified key questions to be discussed by the participants during the workshop. The topics and presenters were as follows:

- Comparison among porewater- and solid-phase-based tests (Steven M. Bay, Southern California Coastal Water Research Project);
- Sediment chemistry, porewater extraction methods: Methodological uncertainties (Bruce Williamson, NIWA, New Zealand);
- Marine and freshwater sediment porewater toxicity testing: Methodological uncertainties and confounding factors (G. Allen Burton Jr., Wright State University);
- Use of porewater toxicity tests in sediment TIEs (Kay Ho, USEPA);
- Use of porewater toxicity tests in SQT studies (Edward L. Long, NOAA);
- Use of porewater toxicity data for the development of sediment quality guidelines (R. Scott Carr, USGS);
- Regulatory applications of porewater toxicity testing (Linda Porebski, Environment Canada); and
- Ecology and the art of misdiagnosis: The need for a paradigm shift (Joe Germano, EVS).

Following the opening talks, the participants were assigned to 1 of 5 workgroups as follows:

- Comparison of porewater and solid-phase sediment toxicity tests;
- Porewater chemistry: Effects of sampling, storage, handling, and toxicity testing;
- Issues and recommendations for porewater toxicity testing: Methodological uncertainties, confounding factors, and TIE procedures;
- Uses of porewater toxicity tests in SQT studies; and
- Regulatory applications of porewater toxicity testing.

Each workgroup was assigned a facilitator and a rapporteur to assist them in focusing and capturing the results of these discussions. Subsequently, each workgroup presented the results of their discussions during a final plenary session. Highlights of the discussions conducted by each workgroup, as well as the recommendations and research needs that were identified, are presented in the following sections of this summary. More extensive information will be provided in the forthcoming book comprised of the workshop proceedings and plenary talks.

## Comparison of Porewater and Solid-Phase Sediment Toxicity Tests Workgroup

Sediment is a naturally occurring particulate material that has been transported and deposited in aquatic ecosystems and is normally found below the water level (ASTM 2000b), including a solid (particulate material) and a porewater phase (water in the interstitial spaces between particles). Sediments provide habitats that support populations of benthic and infaunal organisms, the status of which reflects the quality and health of the ecosystem. The assessment of sediment quality generally involves an evaluation of solid-phase sediments, but pore water is also an important matrix for the assessment of sediment quality, because it represents a major route of exposure to sediment-dwelling organisms (Whiteman et al. 1996; Carr et al. 1989; Adams et al. 1985) and substantially influences the bioavailability of contaminants (Carr, Chapman, Presley et al. 1996; Ankley et al. 1994; Di Toro et al. 1991). Investigation of concordance between porewater and solid-phase toxicity tests provides a tool to evaluate the efficacy (e.g., discriminatory power) and ecological relevance of porewater tests. Because of the complexity of the interactions between contaminants and biota in benthic systems, generalizations about concordance between solid-phase and porewater toxicity tests are difficult. By identifying the causes of agreement/disagreement between tests, much can be learned about the factors that have the greatest impact on the results of porewater toxicity tests.

Complete agreement between solid-phase and porewater toxicity tests is neither required nor necessarily desirable. Porewater toxicity tests are often intended to complement solid-phase sediment quality assessments by providing information on more sensitive species or alternative exposure conditions. Consequently, variable levels of agreement between test methods are to be expected, especially in situations where toxicity is due to moderate or mild levels of contamination. There is no single acceptable level of concordance between tests. Rather, the degree of concordance should be a function of the study objectives and the sample characteristics.

Situations where porewater and solid-phase toxicity tests would be expected to give similar results include studies of sediments from highly-contaminated sites, reference sites, situations where pore water is the primary route of exposure, and studies where the same species is tested in both porewater and solid-phase matrices. Low concordance is commonly observed when there are considerable differences in the relative sensitivities of the different species tested and/or the endpoints used in the tests, when there are different exposure pathways, or when the discriminatory power of the tests differs for naturally occurring chemicals (e.g., ammonia). Effective tools for interpreting test data and the significance of discordance between solid-phase and porewater toxicity test results include the integration of either a TIE or SQT approach into the testing framework.

The flowchart presented in Figure 1 was generated to help interpret the significance of concordance and discordance between porewater and solid-phase toxicity tests. The framework classifies comparative test results on the basis of 3 key characteristics:

- presence or absence of concordance between solid-phase and porewater tests,
- presence or absence of toxicity, and
- presence or absence of contamination.

Classification of the degree of test concordance is the first step in using the conceptual framework. When both porewater and solid-phase sediment tests show no toxicity, no further interpretation from a toxicological perspective is required as the porewater toxicity test results confirm the results of the solid-phase test. When both tests indicate toxicity and this is consistent with the contaminant concentrations in the sediment, no further information is needed to interpret the results. If both tests indicate toxicity and concentrations of contamination in the sediment are below threshold effect levels, the presence of confounding factors or unmeasured contaminants should be investigated.

Differences in responses between solid-phase and porewater tests may require additional evaluations to understand or resolve the underlying factors that are influencing the test results. The following recommendations are provided to enhance the assessment of sediment quality conditions:

Whenever possible, both solid-phase sediment and pore water should be tested, and species with different sensitivities should be included in the testing procedures. Incorporation of these conditions in the assessment enhances the ability to discriminate sediment quality and identify exposure route, and it contributes to the weight-of-evidence approach.



Potential confounding factors in pore water should be identified and measured in order to help interpret test results, understand the contribution of these factors to concordance/discordance between methods, and contribute to TIE procedures.

Whenever possible, contaminants in pore water should be measured, as well as those in solid-phase sediments, in order to provide information on exposure level and routes of exposure, aid in the interpretation of test results, and identify sources of toxicity.

Since a lack of concordance between toxicity tests often occurs, the following research needs and information gaps were identified:

- Naturally occurring porewater characteristics that influence toxicity test results should be identified and acceptable ranges for these variables established.
- The role of porewater toxicity tests should be defined for situations when the aqueous phase is not the primary route of exposure.
- Comparative sensitivities to contaminants of porewater and solid-phase test species should be evaluated.
- The analytical methods for chemical analysis of pore water should be improved.
- Porewater toxicity test methods should be improved, validated, and standardized.

### Porewater Chemistry Workgroup: Effects of Sampling, Storage, Handling, and Toxicity Testing

It is nearly impossible to remove a porewater sample from sediment and expose organisms to it in a toxicity-testing vessel while preventing changes in the chemistry of its natural and anthropogenic organic and inorganic constituents. Although the degree of change in the chemistry of various constituents that occurs in the process of porewater sampling, extraction, manipulation, storage, and testing can be significant, the results of the porewater toxicity tests can be meaningful, as shown by reproducibility of toxicity tests, comparisons with solid-phase experiments, TIE procedures, and comparisons with infaunal surveys. The procedures used for sampling and assessing the chemistry of pore waters and sediments is dictated by the purpose of the study and what question(s) are to be answered (e.g., if the investigation is research-oriented, conducted as part of routine testing, or implemented as part of large-scale monitoring). Several issues related to the effects of sediment porewater sampling, processing, and storage have been discussed in a number of reviews (ASTM 2000a; Burton 1998; Bufflap and Allen 1995; Adams 1991). This section aims to provide guidance on advantages and disadvantages of the various procedures used in sediment porewater sampling, processing, and storage from the viewpoint of how they affect porewater chemistry.

As a general rule, the type of investigation (i.e., what questions are to be answered) will drive several aspects of the sampling design. In situations where the broadest types of questions are being asked (i.e., is this sediment toxic?), the sample collection, extraction, storage, and testing approach is the most difficult to design to ensure that changes in the natural chemistry of the pore water and bioavailability and concentration of chemical contaminants are minimized. Careful consideration has to be given to experimental design issues, including contaminants of concern, site selection, sediment depth of interest, spatial-temporal scale of sampling, erosional versus depositional zones, core versus grab samples, and use of the data (i.e., research, monitoring, or regulation).

Two broad categories of procedures exist for sampling sediment pore water: *in situ* methods, which involve the collection of pore water by the use of samplers (peepers) that are directly inserted into the sediment and left to equilibrate or by suction through the application of vacuum; and *ex situ* methods, where the sediment of interest is removed from the natural setting and the pore water isolated elsewhere, usually by pneumatic pressure or centrifugation, although extraction by vacuum can also be used. The advantages and disadvantages of the different methods of porewater isolation for toxicity testing are summarized in Table 1. A comparison of the various methods of collecting pore water relative to chemical considerations is presented in Table 2.

It is particularly difficult to devise generic guidance for the storage and handling of sediment samples for porewater extraction, due to the fact that porewater extraction and testing often introduce artifacts that are unavoidable (e.g., iron

Table 1 Advantages and disadvantages of several methods for isolating sediment pore water for toxicity testing.

Method	Advantages	Disadvantages
Peeper (in situ)	<p>Porewater chemistry is measured without significant disturbance of the in situ equilibrium conditions.</p> <p>Reduced sample manipulation</p> <p>Reduced sampling influences on the oxidation state of metals</p> <p>Eliminated potential for loss of volatile substances, such as H<sub>2</sub>S, and high Henry's law constant HOCs, which occur with ex situ methods</p> <p>Use of a dialysis membrane eliminates the post-retrieval pore water filtration</p> <p>pH and redox conditions are relatively unaltered, minimizing changes in pH and oxygen-sensitive species (such as metals)</p>	<p>Operates well for inorganic constituents (e.g., divalent metals, NH<sub>3</sub>), but their utility for accurately sampling highly hydrophobic organic compounds is poorly defined (i.e., sorption of hydrophobic compounds onto the sampler, the dialysis membrane, or onto the fouling organisms associated with the membrane, depending on the length of deployment, could artificially reduce pore water contaminant concentrations).</p> <p>An extended equilibration time in the field is required (generally 15 to 20 days), resulting in the need for 2 field trips: 1 for peeper deployment and 1 for peeper retrieval.</p> <p>Sample volumes are limited, generally to less than 10 ml. Larger peepers are limited to very porous substrates.</p> <p>Uncontaminated water inside newly deployed peeper cells could effectively dilute pore water contaminant concentrations in low-porosity sediments.</p> <p>Samples must be collected from peepers immediately upon retrieval, resulting in a longer holding time for pore water outside of its natural matrix prior to toxicity testing.</p> <p>A high degree of technical competence and effort is required for proper use. Use in deeper water requires diving.</p> <p>In situ methods are often not practical for deep waters or high-energy situations</p>
Suction (in situ)	<p>Easy and low-technology operation; use of inexpensive equipment</p> <p>Is suitable for use with a wide variety of sediment textures</p> <p>Procedure can generate large volumes of pore water</p>	<p>Potential sorption of metals and HOCs on 'filter'</p> <p>Some clogging may occur in small-to-medium particle-sized sediments and slow down the porewater extraction process.</p> <p>Collection of pore waters from non-targeted depths (e.g., overlying water) may occur when collection is conducted in situ.</p> <p>Degassing of pore water may occur.</p>
Centrifugation (ex situ)	<p>Several variables (e.g., duration, speed) can be varied to optimize operation</p> <p>Procedure can generate large volumes of pore water</p> <p>Functions with fine-to-medium particle-sized sediments</p> <p>Easy operation</p>	<p>Labor intensive (e.g., sediment loading); requires a refrigerated centrifuge with large tube capacity</p> <p>Lack of a generic methodology</p> <p>Potential sorption of HOCs to centrifuge tube</p> <p>Lysis of cells during spinning</p> <p>Does not function in sandy sediments</p>
Pressurization (ex situ)	<p>Can be used with highly bioturbated sediments without lysis of cells</p> <p>Procedure can generate large volumes of pore water</p> <p>Can be used with a wide variety of sediment textures</p>	<p>Potential loss of HOCs on filter</p> <p>Changes in dissolved gasses may occur</p>

Table 2 Comparison of different methods of isolating pore water for toxicity testing (assuming the use of optimal experimental procedures).

Consideration	In Situ Methods		Ex Situ Methods	
	Peeper	Suction	Centrifugation	Pressurization
Potential for changing chemical equilibrium conditions during sample isolation	Inorganics: Low Nonpolar organics: High	Inorganics: Intermediate Nonpolar organics: High	Inorganics: Low Nonpolar organics: Low	Inorganics: Low–intermediate Nonpolar organics: High
Potential for sample oxidation	Low	Low	Intermediate–high	High
Potential for loss of volatile compounds (e.g., H <sub>2</sub> S, CO <sub>2</sub> , volatile organics)	Low	High	Intermediate	Intermediate
Filtration	Dialysis membrane or screen	Some degree of mechanical screening required	Not necessary	Some degree of mechanical screening or filtering required
Appropriateness for sampling hydrophilic compounds	Good	Good	Good	Good
Appropriateness for sampling hydrophobic compounds	Requires evaluation	Requires evaluation	Reasonable–good	Requires evaluation
Appropriateness for use with ammonia-rich sediments	High	In situ porewater collection: High Ex situ porewater collection: Intermediate	Intermediate	Intermediate
Required level of overall sampling and isolation effort	High	Low–intermediate	Intermediate	Intermediate
Volume of pore water isolated	Small–intermediate	High	High	High
Availability of methodological information	High	Low	High	High
Ability to sample pore water at specific sediment depths	High	In situ: Low–intermediate Field-collected sediment: Intermediate to high	Intermediate–high (if isolated from core sections)	Intermediate–high (if isolated from core sections)
Applicability in a wide range of sediment particle sizes	All	All	Compactable sediments	All

oxide precipitation). The recommendations made herein are intended to minimize changes in porewater composition. Since some changes are time-dependent, as a general principle, toxicity testing should be conducted as soon as possible after sediment collection, i.e., storage times should be minimized. However, due to scheduling and other logistical considerations, toxicity tests often cannot be performed immediately after sample collection; in these cases storage and handling procedures become important concerns. Therefore, 2 options have been used:

- 1) storage of pore water in situ, i.e., store the sediment sample as intact as possible, while maintaining it at 4°C to slow biological/biochemical processes, then extract pore water immediately prior to use; and
- 2) extraction of the pore water and storage at 4°C (short-term) or frozen (long-term).

The method selected for storage (isolated porewater samples or sediment cores/grabs) and the length of storage time may depend, to some extent, on the suspected contaminants of interest. The key principle is that sediment storage time should be kept to a minimum, preferably less than 2 weeks. Short-term porewater storage (ideally, less than 24 hours) should be done at 4°C, in the dark (to minimize bacterial activity and photo-oxidation), under a N<sub>2</sub> atmosphere, with minimal headspace, and in clean vessels with low absorptive capacity for trace metals and hydrophobic organic compounds (HOCs). The freezing of water samples is a complex issue, since it preserves the integrity of a water sample against bacterial decay, but may cause the precipitation of dissolved species such as silica and metal complexes, and alteration of major ion chemistry (Hilton et al. 1997; Tallberg et al. 1997; Adams et al. 1980), although Carr and Chapman (1995) found the toxicity of pore water from the vicinity of petroleum production platforms to be unaltered by freezing.

When pore water is introduced to the test vessel, a cascade of chemical changes may occur. Some of the main changes include oxidation of the anoxic or suboxic porewater sample due to gas exchange between water and the atmosphere and interaction of porewater constituents and contaminants with the testing chamber, organisms, and their food and waste.

Keeping in mind that the purpose of each porewater investigation should dictate the method and procedures used to collect pore water for toxicity testing, that the collection of pore water introduces artificiality to the sample, and that the objective of the extraction procedure should be to preserve the chemical and toxicological characteristics of the pore water as best possible, a consensus on the following conclusions and recommendations was achieved:

- Peepers may provide better information on actual in situ chemistry, at least for inorganic contaminants and major constituents, but may be unsuitable for sampling HOCs due to potential sorption on construction materials.
- Ex situ sampling using centrifugation appears to be most widely applicable for routine porewater toxicity testing and is most suitable for isolating pore water containing HOCs.
- Core or grab sampling devices can be used to collect sediment for porewater isolation, but disturbance and manipulation of the sediment and sediment profile should be minimized prior to porewater extraction. Sediment samples should be stored at 4°C in the dark under nitrogen (or another inert gas) and/or 0 headspace.
- Porewater isolation can be performed in either the field or the laboratory, but the laboratory is generally recommended since the pore water is maintained as close to its original state as possible during transport and storage.
- When pore water is isolated in the field, samples should be stored with the container completely filled and without any headspace, kept cool (4°C), and toxicity testing should be initiated as soon after porewater isolation as possible.
- If porewater samples cannot be tested soon (<48 hours) after sampling, they may be stored frozen.
- For static renewal tests, pore water should not be isolated all at once, but in small batches as needed.
- Easily measured and toxicologically important chemical characteristics, including salinity, pH, conductivity, alkalinity, hardness, DO, NH<sub>3</sub>, H<sub>2</sub>S, and Eh, should be measured and recorded after sample collection and storage. Furthermore, these parameters are expected to change during sample manipulation and toxicity testing.
- To improve accuracy, consideration should be given to using a combination of both in situ and ex situ methods to assess field and laboratory conditions.
- Minimize handling and storage of isolated pore water prior to testing, and initiate toxicity testing as quickly as possible after porewater isolation. Pore water may require aeration in order to conduct the toxicity test.

The most fundamental questions regarding chemical aspects of porewater testing revolve around the limitations of porewater toxicity tests that are imposed by the chemical changes that inevitably occur during porewater testing procedures. These include oxidation of dissolved Fe<sup>2+</sup>, Mn<sup>2+</sup>, HS, and dissolved organic carbon (DOC); precipitation of Fe and Mn oxides and sulfur; adsorption of metals and HOCs; and degassing/volatilization of CO<sub>2</sub>, H<sub>2</sub>S, and volatile hydrocarbons. The following research needs were suggested to advance the science of porewater chemistry in the special context of the toxicity testing of pore waters:

- Develop a standard procedure allowing for the oxidation/precipitation of Fe and Mn oxides and sulfides, along with alterations to DOC, prior to the initiation of toxicity testing. Pore waters are frequently anoxic and oxidation/precipitation reactions are commonly observed. These changes are inevitable in many porewater toxicity tests, so it may be more effective to let these reactions occur in a prescribed manner during the test procedure.
- Investigate the impact of Fe and Mn oxide precipitation on toxicity test results for both metals and organic substances.
- Develop a better understanding of the concentrations and distributions of prominent colloid species in porewater tests, including DOC and Fe and Mn oxides. Specifically, a quantitative understanding of the interactions of colloids with surfaces and with dissolved contaminants is needed.
- Conduct simple aqueous-phase experiments to scope the extent of degassing and volatilization of CO<sub>2</sub>, H<sub>2</sub>S, and NH<sub>3</sub> with sampling, extraction, and storage procedures to provide guidance for method standardization and for assessing the importance of these effects.
- Test the validity of extraction methods using simple systems (i.e., spiked solutions and sediments) to evaluate volatilization and sorption of HOCs, metals, and NH<sub>3</sub> to the apparatus.
- Evaluate the effects of sample holding time on the fundamental constituents in both pore water and whole sediments (e.g., DO, redox, CO<sub>2</sub>, DOC, H<sub>2</sub>S, NH<sub>3</sub>, Fe and Mn).

## Porewater Toxicity Testing Workgroup: Methodological Uncertainties, Confounding Factors, and Toxicity Identification Evaluation Procedures

The chemical changes associated with porewater extraction and sediment handling procedures described in the previous section represent major concerns for porewater toxicity testing and its use for TIE procedures. These changes can affect the equilibrium of contaminants contained in the sediment and the pore water, which affect the toxicity in the pore water and solid-phase sediment alike (see Burton 1992 for a review; Environment Canada 1994).

In situ porewater extraction by peepers was identified as the method that causes the least disruption of natural sediment conditions, and therefore would be the method of choice for the extraction of pore water to be used in toxicity testing; however, the practicality of toxicity testing must also be considered. Peeper methods often do not yield enough pore water for toxicity testing and/or TIE manipulations, therefore large-scale centrifugation or squeezing is often the only practical method of porewater extraction. If surveys are conducted in shallow areas, or if relatively undisturbed sediment cores or grabs can be collected, vacuum extraction of pore water can be conducted on site (Winger and Lasier 1991). This would cause minimal disruption and allow the collection of large enough volumes of pore water for use in toxicity tests and TIE procedures (Winger et al. 1998). Although in situ porewater extraction is recommended as the preferred method, for the sake of consistency and comparability only 1 porewater extraction method should be used throughout a single survey, no matter which method is chosen. The toxicological implications of chemical changes caused by porewater extraction methods are summarized in Table 3.

Porewater storage time and conditions can also introduce a series of artifacts that can considerably affect the toxicity of pore waters; this is clearly an area that needs more research. Although it is recommended that porewater samples should be extracted and tested as soon as possible, this is often not practical, and therefore, we need to balance our concern for minimizing changes with practicalities. No consensus on appropriate methods to store pore waters exists; 1 school of thought is that sediments should be stored at  $4 \pm 2^\circ\text{C}$  and pore water extracted within 24 hours of toxicity testing (Environment Canada 1994), while the other school believes that pore water can be extracted immediately after sediment collection and frozen after removal of particles without changing toxicity (Carr and Chapman 1995). In all cases, the interpretation of results should consider the porewater storage conditions and duration.

In addition to uncertainties associated with porewater extraction and storage, several characteristics of sediments (such as salinity for marine and estuarine pore waters, hardness and alkalinity (Lasier et al. 1997) for freshwater samples, DO, pH, and numerous others) can affect the results and act as confounding factors in porewater toxicity tests. In this context, a confounding factor is considered to include any substance or physico-chemical variable that can mask or interfere with the results and/or interpretation of a porewater toxicity test. The classification of a substance as a confounding factor is dynamic, and it may change depending upon the objectives of the assessment. Therefore, a substance may be a confounding factor in some situations and a contaminant of concern in others. For instance, ammonia is naturally occurring (Chambers et al. 1992) and may cause toxicity in pore waters from uncontaminated sediments. In some cases, however, ammonia may be directly introduced into the environment or increase as a result of anthropogenic activities. Examples include ammonia-bearing effluents and wastewater discharges, organic or nutrient enrichment, or reduced bioturbation due to anthropogenically induced death of benthos. The most commonly recognized confounding factors are salinity (for marine/estuarine water), conductivity, hardness and alkalinity (for freshwater), DO, total and dissolved organic carbon, pH, ammonia, and sulfide, but there are many other organic and inorganic constituents and physico-chemical parameters in pore water which may be important. Confounding factors may affect porewater toxicity test results by their presence at a concentration that is directly toxic to the test organisms, for which reason it is important that the tolerance levels of the test organism be determined. Alternatively confounding factors may affect the bioavailability of contaminants or change them to a toxic form. For instance, pH, temperature and salinity affect the toxicity of ammonia; pH and hardness affect the toxicity of many metals by transforming them into more or less toxic species, and DOC affects bioavailability of organics and some metals. Since organisms will only tolerate a given range for confounding factors, outside of which toxicity testing is impractical, guidance is provided in toxicity test protocols for adjustment of factors such as temperature, DO, and hardness or salinity.

The sediment depth to be sampled for porewater extraction is dependent upon the objective of the study and should be considered on a case-by-case basis, in order to match the expected exposure of organisms in a real-world situation. If

Table 3 Toxicological effects of chemical changes induced by porewater extraction.

Extraction Method	Methodological and Chemical Factors	Effect on Toxicity or Testing Conditions
Peeper	1) Reduced sample manipulation; 2) No significant disturbance of the in situ equilibrium conditions; 3) Elimination of potential for loss of volatile substances that occurs in ex situ methods; 4) Elimination of need for post-retrieval porewater filtration when dialysis membrane is used, which minimizes loss of some contaminants; 5) Relatively unaltered redox and pH conditions, which minimizes changes in pH and oxygen-sensitive species (such as metals). Minimal changes in the oxidation state of metals	Realistic assessment of toxicity  Realistic assessment of toxicity when metals are contaminants of concern
	Sorption of highly hydrophobic organic compounds onto the sampler or to associated fouling organisms, if present	Underestimation of actual toxicity
	Uncontaminated water inside newly deployed peeper could dilute porewater contaminant concentrations in low-porosity sediments.	Underestimation of actual toxicity if equilibrium is not reached
Suction	Sorption of metals and organochlorinated HOCs on filtering medium Collection of pore waters from non-targeted depths as well as overlying water may occur when collection is conducted in situ Degassing of pore water may occur	Underestimation of actual toxicity  Actual toxicity can be under- or overestimated, depending on quality of overlying water and pore water at different depths Toxicity may be underestimated due to loss of volatile organic compounds
	Several variables (e.g., duration, speed, temperature) can be varied	Lack of a generic methodology makes comparisons of toxicity data from different surveys difficult
Centrifugation	Sorption of HOCs to centrifuge tube depending on construction material Does not function in sandy sediments	Underestimation of actual toxicity
	Lysis of cells can occur during spinning, releasing DOC	Prevents toxicity assessments in several critical areas that are typically sandy Released DOC can bind contaminants, causing underestimation of actual toxicity
Pressurization	Can be used with highly bioturbated sediments without lysis of cells	Prevents DOC release from broken cells and consequently allows realistic toxicity assessment
	HOCs loss on filter	Underestimation of actual toxicity

porewater toxicity is to be assessed for dredging purposes, the depth to be dredged should be sampled for porewater extraction and toxicity analyses, but in a field survey intended to assess exposure of benthic biota, only the depth that is inhabited by benthic organisms should be sampled. Local dynamics, severity of storm events and consequent perturbation, and other similar factors should also be considered during study design.

Due to the difficulty in obtaining pore waters, test species that use small sample volumes are most amenable for testing. Species and test endpoints currently used in porewater toxicity tests throughout the world were discussed by Carr (1998). Different types of porewater toxicity tests encompass a variety of species from different habitats (e.g., planktonic vs. benthic), life stages, endpoints, as well as in situ and laboratory tests. Different endpoints and organisms are required to answer different questions, and the researcher should consider this when selecting test organisms. Understanding the sensitivity of different organisms allows the design of experiments that better address the objectives of a particular study. Although in some limited cases a species indigenous to the area of the sample collection might be required, in most situations it is not necessary to use organisms indigenous to the area of sample collection. Particularly, when a non-benthic

organism is being used in the test, there is little to be gained from a scientific standpoint by requiring the use of an organism native to the area. Most porewater toxicity tests currently in practice are acute exposures and there is a need to develop a broader range of short-term chronic tests with pore water.

Porewater toxicity tests can be conducted in the laboratory or in situ, and there are benefits and limitations associated with both. In situ tests have the advantages of providing exposures that are dynamic and integrated to potentially important environmental factors (suspended solids, suspended organic matter, sunlight, flow, biogeochemical gradients) and can be successfully conducted with a number of species and life-stages. However, limitations of in situ tests include: need for an equilibration period after chamber deployment and on-site acclimation of test organisms, methods are not standardized and have less historical use, deployment and retrieval of chambers may be difficult or impossible in some environments, and there is little control over treatment variables. Both laboratory and in situ testing, however, provide unique information that contributes to the weight of evidence in environmental assessments.

The use of pore water in TIE procedures is a key application when causality is an issue of concern, since TIE methods are not yet fully developed for solid-phase sediment tests. Currently, only TIE methods for pore waters are routinely used to identify stressors in sediments. In general, TIE methods used for effluents and pore waters are similar, with the exception of the closed chamber manipulation used to maintain pH in the graduated pH method. In pore waters, the chemical oxygen demand of the sample, combined with the biological oxygen demand of the organisms may decrease DO concentrations below those required by the test organisms. The same uncertainties and confounding factors that affect porewater toxicity testing will affect porewater TIE procedures, including changes in the bioavailability of toxicants during collection, extraction, or storage, as well as exposure of organisms to concentrations of contaminants that are unlike field exposures.

The following recommendations for the study design for porewater toxicity surveys are summarized in Figure 2:

- The use of in situ peepers to collect pore water is recommended whenever it is feasible. The second option is sampling pore water in situ by vacuum. If in situ sampling is not practical, whole sediment samples should be collected by cores, dredges, or grabs, stored at 4°C for the minimum possible amount of time, and pore water extracted as close as practical to the time of the toxicity test. It has also been demonstrated that in several cases freezing pore water after removal of particulates does not significantly affect its toxicity and, therefore, freezing after removal of particulates by centrifugation (not filtration) is also considered an acceptable option.
- Centrifugation, vacuum, or pressurization are suitable for ex situ porewater extraction, since they provide relatively large amounts of pore water. The method of choice is dictated by logistics and sample characteristics. Centrifugation is recommended if organics are the primary contaminants of concern.
- The sediment sampling depth should match the expected exposure of organisms in the field, and should reflect the objective of the study (e.g., dredging, assessment of potential impact on benthic biota) and/or on dynamics of the system to be surveyed (e.g., prone to storm events that disturb sediment to extensive depth, coarse grain size, and high currents).
- Filtration of pore water is not recommended. Pore water should be double-centrifuged if centrifugation is the selected extraction method, or centrifuged after other methods of extraction have been applied.
- The same porewater extraction method should be used throughout a survey, regardless of which method is selected.
- Most marine/estuarine test methods recommend adjustment of the salinity of porewater samples to within 1 or 2 parts per thousand within the test organism's physiological requirements to control the effect of this variable on the test results.
- Ammonia and sulfides should be measured in porewater samples submitted for toxicity testing and the tolerance of the test organisms to un-ionized ammonia and sulfide should be established for comparison to the levels measured in the samples.
- In addition to ammonia and sulfides, the following porewater characteristics should be measured: DO, pH, salinity (marine and estuarine samples), hardness, alkalinity and conductivity (freshwater samples), Eh, TOC, and DOC, as well as nutrients in samples used for phytotoxicity tests.
- The use of indigenous organisms in laboratory tests is seldom necessary, unless there is a particular species of concern in the study area.
- Some species may be invasive and should not be used for in situ testing unless they are resident species. The use of water column organisms for porewater toxicity tests is considered scientifically appropriate.

Figure 2 Optimizing sediment and porewater sampling design

Project Design Question: *Is there a need for porewater chemistry or toxicity information?*

Yes, if: Need to verify effects based on sediment quality guidelines (EqP);  
 Need to build a weight-of-evidence based conclusion;  
 Advantageous to assess the more bioavailable fraction of sediments (i.e., pore water);  
 Advantageous to use standardized water toxicity assay methods;  
 Advantageous to apply TIE methods;  
 Upwelling, downwelling, or dynamic porewater conditions exist (that do not allow for accurate assessment of sediment quality/risk based on chemistry or whole sediment toxicity testing).

Objective of survey:

Monitoring

*Are peepers feasible?*

Yes, if: Shallow – accessible for manual deployment;  
 Minimal porewater volumes needed;  
 Expertise available;  
 Sediment depth of concern can be matched with peeper exposure;  
 Equilibration time can be met.



Yes



Oxic Sediment

*In situ* organism exposure

Options: Toxicity testing 1 to 4 d;  
 Bioaccumulation 1 to 4 d.

Mesh size: dialysis to 150 μm  
 depending on questions and  
 sediment contaminants.

*NOTE: Certain plastics and small mesh membranes may sorb organics; larger mesh membranes allow initial entry of colloids and particulates. Consider effects of these factors vs. project questions and objectives.*

**IMPORTANT MEASURES FOR MOST POREWATER SITES:**

Porewater flow, TSS, DO, pH, Temp., NH<sub>3</sub>, Sulfide, Salinity, TOC  
 Alkalinity, Hardness, Conductivity, + Chemicals of Concern (COCs)

*NOTE: Attempt to match sampling depth with biota exposure depths. Minimize oxidation. Minimize processing manipulation and time until analyses/toxicity testing.*

Anoxic Sediment

Equilibrate 2 to 14 d depending on  
 chamber size/mesh pore size,  
 sediment;  
 Pre-purge chambers with nitrogen  
 prior to deployment.

Dredging

Use grab or core samplers, collect sediment  
 throughout the whole depth to be dredged,  
 homogenize and extract pore water using  
 laboratory method of choice, and conduct  
 laboratory porewater toxicity tests.



No: *Are Corers feasible?*



Yes, if: Sediment unconsolidated, mainly clay/silt;

Appropriate equipment, logistical support  
 and time available.

*NOTE: Use least destructive coring method possible. Match sampling depth with biological effect zone. Use in situ core suction if available.*

No: Use Grab sampler.

*Note: Use least destructive grab method available, e.g., Ekman > Ponar > Van Veen*

- Appropriate reference sites should be selected from a location near the study site, or at least in the same ecoregion, and have similar sediment (and therefore porewater) characteristics.
- If confounding factors are manipulated (e.g., TIE), caution should be exercised to avoid removal of contaminants of concern. As a general rule, TIE manipulations should not be performed separately, but as a suite.

To improve the understanding of the effects of methodological variables, confounding factors, and TIE manipulations on the results of porewater toxicity tests, research on the following aspects is needed:

- Determine acceptable sediment storage time and conditions prior to porewater extraction.
- Evaluate the effect of porewater extraction and storage methods on microbial processes such as degradation of contaminants and ammonia production.
- Further evaluate the effect of freezing and other storage methods on chemistry and toxicity of contaminants contained in pore water.
- Develop improved (larger-volume) in situ porewater collection devices.
- Assess different ways of introducing oxygen into samples while minimizing changes to the pore water and contaminants contained in it.
- Evaluate the effect of the oxidation of metals and organics on porewater toxicity.
- Determine the effect of the sorption of contaminants to test and storage vessels and the relation of sorption to container type and surface area to water volume.
- Determine the tolerance of test organisms to a variety of confounding factors, e.g., ammonia, sulfides, pH.
- Determine the relationship between porewater tests and field conditions to help assess the utility of porewater testing and TIE procedures.
- Determine appropriate porewater sample volumes, according to the test species, to maintain test exposure conditions (e.g., hydrophobic contaminants, DO, metabolic waste).
- Develop a broader range of short-term chronic tests in small volumes for use with porewater tests.

## Uses of Porewater Toxicity Tests in Sediment Quality Triad Studies Workgroup

The SQT approach to sediment quality assessments was born out of the realization that integrated information was necessary to accurately portray the relative quality of sediments (Long and Chapman 1985). The SQT approach relies upon the analyses of data from chemical analyses, toxicity tests, and infaunal benthic structure assessments, which can be used by sediment assessors to form a weight of evidence to compare and rank the relative quality of sediment samples and regions of a study area (Long 1989; Chapman et al. 1987). Multiple toxicity tests commonly are applied as a part of SQT studies and guidance is available on how to use these data in judging the relative toxicity of sediments (Chapman et al. 1997). Use of a single, unitless index to portray the toxicity of sediments is not encouraged; rather, the use of the data from all individual test results has been recommended (Chapman et al. 1997). Thus, the toxicity data from tests of pore waters should be used along with the parallel data from tests of other sediment phases to form a weight of evidence and to determine concordance among the triad components.

Although toxicity tests of pore waters have been used primarily in large-scale (e.g., estuary-wide) triad surveys (Carr, Chapman, Howard et al. 2000, 1996; Fairey et al. 1998), they can be used for other related purposes. Most applications of these tests in SQT studies involve various kinds of determinations of spatial status and temporal trends. The types of hypotheses tested, the kinds of tests selected, and the methods used during data interpretation are dependent upon the objectives of the study.

Seven possible applications of porewater toxicity tests in triad studies include, but are not restricted to:

- identifying spatial trends in sediment quality within a specified study area (i.e., an assessment of status and trends);
- determining the magnitude (e.g., degree and severity) of degraded sediment quality;
- identifying and justifying designation of hotspots with unacceptable sediment quality;
- determining sediment quality adjacent to a designated upland waste site (e.g., off-site migration of contaminants);
- determining the biological significance of known contamination;

- determining temporal trends in sediment quality within a specified area; and
- development of SQGs or field validation of their predictive ability.

Within the SQT, a suite of different toxicity tests should be used to reduce the uncertainty and limit the occurrence of false negative and positive results. Several attributes of porewater toxicity tests are desirable for SQT studies, including insensitivity to confounding factors, ecological relevance, sensitivity to target contaminants of concern, and a range in response that would allow differentiation among samples that may all be highly contaminated or all moderately contaminated. Based on the desired attributes, some porewater toxicity tests were identified as the most appropriate for use in SQT studies. Early life history tests with sediment pore water allow greater discrimination among sites, therefore, embryo/larval stages of mollusks, polychaetes, crustaceans, echinoderms, and fish are potential candidates for porewater testing. The use of organisms that occupy a particular trophic level can be useful for the characterization of sediment porewater toxicity, i.e., the use of a primary producer (freshwater or marine algae) and a primary consumer (freshwater or marine invertebrates) allows for the determination of the most sensitive trophic level for a particular environment. Porewater toxicity testing may be an order of magnitude more sensitive than whole-sediment toxicity testing, which allows for further investigations for those sediments that may be producing more complex changes to the benthic community (Carr et al. 2000; Carr, Chapman, Howard et al. 1996; Carr, Long et al. 1996; Carr and Chapman 1992).

The main challenge facing investigators who have applied the SQT approach to aquatic impact assessment over the past 2 decades is how to interpret the array of data from the different measurement endpoints (sediment chemistry, benthic community structure, and toxicity tests). There have been numerous suggestions put forth in the literature, including summary indices (Chapman 1992a, 1990; Alden 1992), tabular decision matrices (Carr, Chapman, Howard et al. 1996; Carr, Long et al. 1996; Chapman et al. 1996; Chapman 1992b), scaled ranking factors (Carr et al. 2000; Canfield et al. 1996; Carr, Chapman, Howard et al. 1996), or multivariate analyses (Chapman et al. 1996; Green and Montagna 1996; Green 1993a, 1993b; Green et al. 1993). All of these approaches require an appropriate reference station or group of stations, and all of these approaches also involve a "weight of evidence" (WOE) interpretation, or a way to draw conclusions based on congruent or conflicting lines of evidence. As with multivariate techniques for data analysis where there is no single "right" way to relate sets of variables (Green 1993a), a recurring theme in these papers (Chapman et al. 1997; Chapman 1996) is that there is no single "best" way to depict or use the SQT. While some investigators may experience greater intellectual freedom employing the SQT because "it is neither constrained nor defined by any particular method of data analysis or presentation" (Chapman et al. 1997), investigators still need to exercise caution in both their sampling design and data interpretation methods.

Before any toxicity tests are routinely included as part of SQT investigations, these tests should be field-validated as a predictive tool by accurately assessing covariation (Germano 1999; Arkes 1981), determining if their inclusion increases diagnostic accuracy (Faust 1986a), and, finally, determining if the information they are providing is redundant with another variable (Faust 1986b). Different combinations of results are possible when SQT studies apply urchin fertilization tests of pore waters, microbial bioluminescence tests of organic solvent extracts, and amphipod survival tests of solid-phase sediments. If significant results are observed in all tests performed, toxicity easily can be scored as a "+" in Chapman's (1996) decision matrix (Table 4). Similarly, if all results are negative, a score of "-" would be justified. If results are significant in 2 of the 3 tests, a "+" score also may be justified based upon the judgment of the sediment assessor, especially if the results are considerably different from controls in both tests showing toxicity. A sample indicating toxicity in more than 1 porewater concentration should be scored higher (more toxic) than 1 in which toxicity was observed only in 100% porewater. When only 1 of a suite of tests indicates toxicity, the sediment assessor will have to apply best professional judgment based on the test species, the degree of difference from the reference, and other factors in the final determination as to whether the sample should be considered toxic in the decision matrix.

Several different spatial patterns can occur in SQT studies, including a general lack of degraded conditions, highly localized areas of degraded conditions, spatial gradient of increasing sediment quality with increasing distance from sources, patchy patterns of degraded conditions, or widespread distribution of degraded conditions. These patterns are likely to be a function of proximity to sources and a combination of physical processes that result in dispersal and deposition of toxicants (Table 4).

Understanding the mechanisms responsible for the expression of porewater toxicity endpoints would be advantageous to

Table 4 Tabular decision matrix for sediment quality triad data (from Chapman et al. 1996) \*

Chemistry	Toxicity	Benthos	Possible Conclusion
+	+	+	Strong evidence for pollution-induced degradation
-	-	-	Strong evidence against any pollution-induced degradation
+	-	-	Contaminants not bioavailable
-	+	-	Unmeasured contaminant(s) or confounding factor(s) have the potential to cause degradation
-	-	+	Alteration is not due to bioavailable contaminants
+	+	-	Contaminants are bioavailable but in situ effects are not demonstrated
-	+	+	Unmeasured bioavailable contaminants or confounding factor(s) are causing degradation
+	-	+	Contaminants are not bioavailable, alteration is not due to bioavailable contaminants

\*Reprinted from *Marine Pollution Bulletin*, Volume 32, PM Chapman, MD Paine, AD Arthur, LA Taylor, "A Triad Study of Sediment Quality Associated with a Major, Relatively Untreated Marine Sewage Discharge", pages 47-64, ©1996, with permission from Excerpta Medica Inc.

aid in identifying the specific toxicants that may be contributing to the measured response. The TIE procedure, which involves a stepwise process of testing and analysis of chemical characteristics to identify potentially causative toxicants, is currently the best method available for determining causality using porewater toxicity tests (Burgess et al. 1996). Ideally, the optimum assessment procedure would involve identification of the specific chemical of concern after an initial screening. Non-chemical-specific assays that are currently in use could be utilized as exploratory tools to identify toxicity. Single chemical test endpoints could then be applied to identify the chemical(s) responsible for the toxicity and to infer whether the community degradation is likely to be a result of a particular chemical or source.

The following conclusions were reached:

- Porewater toxicity tests may be able to be used to satisfy a number of different objectives in SQT studies, mostly involving assessments of status and trends.
- Toxicity in porewater tests, together with toxicity in other tests, elevated chemical contamination, and altered infaunal populations, can be used to classify sites as "degraded."
- Observations of toxicity in porewater or whole sediment toxicity tests alone may not constitute a sufficient weight of evidence to classify sediments as degraded.
- Often, toxicity in porewater tests occurs in samples with levels of measured chemical contamination insufficient to cause toxic responses in other tests.
- Only a small fraction of the chemicals and their degradation products that occur in the complex mixtures of contaminants typically found in urbanized estuaries are included in the "comprehensive" chemical analyses.
- The factors that control the bioavailability of sediment-associated contaminants are not completely understood.
- Concordance between the porewater toxicity data and sediment chemistry is most likely to be observed in studies where there is a wide range in contaminant concentrations and a wide range of responses in porewater toxicity.

This workgroup recommended that toxicity tests need to be assessed for covariation and base rates in order to better determine their value for making predictions about effects to the benthos. The following recommendations were also endorsed by the group:

- Samples determined to be toxic in dilutions less than 100% pore water should be classified as more toxic than those determined to be toxic only in 100% pore water.
- A multivariate analysis is the best statistical approach to demonstrate concordance among SQT components.
- SQT studies should be designed to maximize the number of stations, even if some reduction in the number of replicate samples is necessary.
- Confounding factors are best accounted for by normalization in univariate tests and by simply including the confounding variables in a multivariate analysis.
- Field sampling programs should follow good design principles and include synoptic sampling for triad components.
- Some researchers recommend using a suite of tests utilizing different species and endpoints to reduce uncertainty and help identify causality. Other researchers caution that using multiple tests which have not been shown conclusively to be accurate diagnostic predictors of environmental effects will only serve to increase uncertainty.

Several research needs were identified for the improvement of the use of porewater toxicity tests in SQT studies:

- More synoptic porewater chemistry and toxicity data is needed.
- A better understanding of the relationships between concentrations of chemical mixtures in sediments and pore water and porewater toxicity is needed.
- An index to summarize benthic data that is easy to understand and not easily misused is needed.
- Generalized multi-parameter patterns in ecological structure and function (as opposed to species differences in different locations) relative to porewater toxicity needs to be determined.
- Development of chemical-specific porewater toxicity tests would be desirable.
- Research on the toxicological mechanism at the cellular level for porewater test organisms is needed.
- Perform the necessary validation studies with sediment toxicity tests to demonstrate that these are indeed valid, accurate metrics.

## Regulatory Applications of Porewater Toxicity Testing Workgroup

In the regulatory arena, decisions are often based as much on legal and programmatic considerations as they are based on scientific considerations. The objective of this discussion group was to evaluate the use of porewater toxicity tests in regulatory applications, including their potential use in the development of SQG values. The following discussion focuses on the appropriateness and readiness of porewater tests for use in regulatory applications, as well as important factors that must be considered prior to porewater toxicity test use in these applications.

The selection of a test must be driven by either the regulatory context in which the results are to be used or the question that is being asked. This admonition also applies to the choice of an appropriate sampling design, reference sediment, and test endpoints. Factors to consider include whether dietary uptake is important, if the test will be used as a stand-alone test or as part of a tiered assessment, and whether particular porewater constituents are considered confounding factors or contaminants of concern.

One of the principal reasons for using porewater toxicity testing in a regulatory framework is that these tests provide additional information not currently provided by solid-phase, elutriate, or sediment-extract tests, as well as the fact that there are more aqueous-phase tests and test species available for sublethal or chronic effects measurement in pore water than there are epifaunal or infaunal test species for equivalent solid-phase tests. Another advantage of porewater tests is that they are generally cheaper and more rapid to conduct than solid-phase tests, which is particularly important in a regulatory context because cost and ease of test performance are important considerations for both testing laboratories and regulated entities.

Potential regulatory uses for porewater toxicity tests include screening, compliance testing, application of TIE procedures, and environmental monitoring and assessment programs. At this time, however, porewater tests are not seen as replacing solid-phase tests or being used alone in pass/fail-type decisions. The use of porewater toxicity testing alone is likely appropriate only for certain screening applications or TIE procedures. In all other applications, the purpose of the investigation or nature of the questions of interest will require the use of additional toxicity testing approaches or higher-tier biological analyses (e.g., solid-phase tests, community assessments).

Table 5 Potential regulatory applications of porewater toxicity tests

Types of Regulatory Programs	Potential Regulatory Application	
	Action Limits	Monitoring
Aquatic sediment removal (e.g., dredging and disposal, subaqueous sand/gravel mining)	?	
Mine decommissioning (e.g., closure plan and sediment reclamation)		?
Sediment remediation programs	?	?
New chemical registration programs (sediment spiking)	?	
Effluent point source controls/cumulative impact assessments (e.g., watershed management, non-point source monitoring)	?	?
Sediment quality criteria		?
Disposal site monitoring		?
Environmental quality monitoring		?
Impact assessment of existing operations (e.g., sediment monitoring of aquaculture activities)		?
Environmental assessment programs (e.g., environmental assessments, environmental impact statements)		?
Fish habitat protection programs		?

Table 5 summarizes regulatory programs that could use porewater toxicity testing data to develop action limits or in monitoring programs. Action limits are defined as specific “pass/fail” levels that may trigger particular actions such as additional testing or studies, or contribute to decisions concerning issuing of permits.

Whether certain sediment characteristics are considered contaminants of concern or confounding factors is a serious issue in the regulatory arena. Sometimes a particular chemical in the pore water (e.g., ammonia) may not be a contaminant of concern for regulatory purposes, but can influence the outcome of a porewater toxicity test. In other situations, however, it may be a contaminant of concern. Some examples of porewater features that may be considered confounding factors in some situations but contaminants of concern in others are given in Table 6. Similar issues involving confounding factors must also be considered when choosing appropriate reference samples for porewater toxicity tests.

There are several categories of criteria against which a porewater toxicity test must be evaluated before a method is ready for regulatory use, including but not limited to:

- sensitivity,
- degree of standardization,
- usefulness,
- ecological relevance, and
- logistical considerations.

A distinction needs to be maintained between regulatory methods and standard guides or research methods. Regulatory methods must be definitive and standardized so they can be used to generate data which support regulatory actions that may be challenged in the courts. Standard guides for porewater toxicity, while useful for general monitoring purposes, may not be sufficiently prescriptive or implemented uniformly enough to survive legal challenge. Published standard test methods currently available for porewater toxicity testing in regulatory applications include the bacterial luminescence and

Table 6 Potential situations where confounding factors may be of concern

Confounding Factor	Potential Situation
NH <sub>3</sub>	Aquaculture, WWTP*
H <sub>2</sub> S	WWTP
Low DO	Aquaculture, WWTP
pH	Acid mine drainage
Ion imbalance	Oil and gas productions

\*WWTP = wastewater treatment plant

the sea urchin and sand dollar fertilization tests. However, laboratory accreditation and certification programs for toxicity testing are typically directed towards the performance of effluent toxicity testing, and criteria that aid in judging the performance and quality of regulatory testing are not currently focused on assessing the ability of laboratories to conduct porewater toxicity tests.

In addition to being used directly to assess sediments in a regulatory context, porewater tests can contribute to the development and verification of other assessment tools such as effects-based SQGs. Sediment quality guidelines are generally divided into 2 groups: empirical guidelines such as ERL/ERM, TEL/PEL, AET values (Neff et al. 1986; USEPA 1989; Long and Morgan 1991; Jaagumagi 1992; MacDonald et al. 1992; Reynoldson and Zarull 1993; Long et al. 1995) and theoretical, equilibrium partitioning (EqP) guidelines such as AVS-SEM (acid volatile sulfides-simultaneously extractable metals)-based guidelines for metals and Equilibrium Partitioning Sediment Guidelines (ESGs) for non-ionic compounds (Di Toro et al. 1991).

The empirical methods are based on large databases of synoptic chemistry and biological effect data. A decision to systematically incorporate porewater toxicity test data into databases used to derive empirical solid-phase SQGs must be based on an understanding of what the numbers will represent and, more importantly in a regulatory context, how they are likely to be used to make decisions. The available data on the effect of inclusion of porewater data on existing guidelines is limited, and the ecological and environmental relevance of either incorporating porewater data into current SQGs or creating separate SQGs using porewater data only is unclear at present.

Equilibrium partitioning sediment guidelines are based on the equilibrium partitioning theory and attempt to predict biological effects based on knowledge of the water-only toxicity of compounds or classes of compounds and their chemical partitioning in sediments. Most of the supporting evidence for the ESG approach has come from solid-phase toxicity tests (as opposed to porewater toxicity tests), but an important part of the EqP theory relates to the correlation of porewater chemical concentration and biological effects (Di Toro et al. 1991), and therefore the potential exists to use porewater toxicity test results to help verify theoretical solid-phase SQGs. The utility of porewater toxicity tests in guideline development and the appropriateness of these approaches for regulatory applications will become more evident as more research is conducted on both SQGs and porewater toxicity tests.

The group generated the following conclusions and recommendations:

- There is a need to determine, a priori, the purposes for using porewater toxicity tests in a specific regulatory program. Regulatory authorities must determine what questions they are trying to answer and ensure that the questions are appropriate for the specific regulatory application.
- Porewater testing can often provide “value-added” information which complements whole sediment, elutriate, and sediment-extract tests. It was suggested that it is worthwhile to pursue the development and standardization of appropriate porewater toxicity tests for use in regulatory programs.
- Advantages for the use of pore water as a test phase in a regulatory context include:
  - direct contact with a sediment fraction (vs. elutriate);
  - ability to use/adapt small water-based tests with developed methods;
  - accessibility to a wider range of sublethal endpoints;
  - fast, low cost, simple, commercial use: screening;

- can use TIE methods more easily;
- can do dilution series;
- less manipulative than solvent extracts (no residual solvent concerns); and
- allows assessment of dissolved phase of sediment (e.g., route-of-exposure information).
- Constraints for the use of pore water as a test phase in a regulatory context include:
  - small volume;
  - artifacts due to sampling, extraction, and storage (oxidation changes, etc.);
  - shelf life;
  - short-term tests;
  - higher frequency of reported sensitivity to contaminants such as ammonia; and
  - methods less developed and standardized.
- Porewater toxicity tests were considered suitable for the following types of frameworks:
  - use alone in rapid screening of sediment toxicity;
  - as a lower tier; and
  - as an integrative tool in a battery of tests, which includes whole-sediment toxicity tests: porewater toxicity testing can be useful in this context because it can provide rapid, cost effective estimates of acute and sublethal responses, genotoxicity potential, and possibly endocrine disruption responses in a tiered approach including other testing or monitoring.
- Porewater toxicity tests would not be suitable in the following regulatory contexts:
  - as a stand alone pass/fail test without corroborating weight of evidence from other support criteria, including chemistry, solid-phase toxicity tests, and/or evidence of effects on benthic communities;
  - as a substitute for a solid-phase test;
  - the use of 2 negative porewater controls (i.e., reference and performance controls) is recommended as in solid-phase sediment toxicity testing;
  - where a suitable reference sediment for porewater extraction and testing cannot be found, the performance control can be used for the statistical comparison for determining which samples are toxic; knowledge of the tolerance levels of the test species to major confounding factors is advisable in this situation;
  - it is important that any test slated for use in a “pass/fail” mode be standardized as a formal testing procedure;
  - the incorporation of porewater toxicity test results into SQGs might generate guidelines that are more predictive of porewater effects and better explain the results of porewater tests. They might also be more protective if, for example, the porewater toxicity tests were to pick up a biological response to a chemical or class of chemicals that was missed by the solid-phase testing; and
  - EqP-derived SQGs might benefit from further evaluation provided by additional synoptic porewater chemistry and toxicity data.

Among the identified information gaps was the fact that additional studies comparing porewater chemistry and toxicity data are necessary, since at the present time it is not clear if the addition of porewater toxicity testing data to the databases used to develop solid-phase SQGs would be valuable. Additional identified information gaps include:

- lack of information on the sensitivity of test species to some confounding factors (e.g., H<sub>2</sub>S).
- lack of standard, published methods for porewater testing.
- lack of laboratory accreditation and certification programs for the laboratories conducting porewater tests.

## Summary and Conclusions

As expected, several of the discussions conducted by the different workgroups overlapped. The chemistry and toxicological methods workgroups addressed the same aspects of porewater testing, but from a different point of view. The SQT and the regulatory workgroups approached the aspect of the use of porewater tests for the derivation of SQGs, but from different angles. The solid-phase and porewater testing comparison workgroup approached the confounding factors issue, which was also extensively discussed by the toxicological methods workgroup, and the regulatory and toxicological methods

workgroups discussed the needs and appropriateness of different reference sites and samples. Rather than indicating redundancy, this shows how all subjects are tightly knit together, how the different fields of science are interdependent, and how important it is to have a multi-disciplinary team for the application and interpretation of porewater toxicity tests.

Several information gaps and research needs were identified by the different workgroups to improve the accuracy and enhance the understanding of porewater toxicity test results. Some of the fundamental needs pointed out were related to improving the understanding of the toxicological effects of the artifacts and changes introduced when pore water is extracted, the effects of porewater storage methods and time, the effects of naturally occurring porewater characteristics that influence toxicity test results (confounding factors), the validation and standardization of porewater toxicity test methods as well as the need for more short-term chronic test methods, the need for more synoptic porewater chemistry and toxicity data for better understanding of the relationships between concentrations of chemical mixtures in sediments and pore water and porewater toxicity, and to identify the most appropriate benthic indicator species as well as the best toxicity endpoints for predicting impacts on the benthos.

Some concluding remarks:

- Concordance between the results of solid-phase and porewater toxicity tests should not always be expected, and discordance is indicative of different routes of exposure and/or species sensitivity, rather than inaccuracy in the results of 1 type of test.
- It is important to conduct both porewater and solid-phase tests whenever possible, which enhances the ability to discriminate sediment quality.
- The toxicity data from porewater tests should be used along with the parallel data from tests of other sediment phases to form a weight of evidence and to determine concordance among the triad components.
- Sampling, extraction, and storage techniques are critically important for achieving the most field-representative samples of pore water. Several sampling methods were suggested, and method selection should be based on the objective of the study.
- It is nearly impossible to avoid artifacts and chemical changes when removing pore water from sediment and using it in a toxicity test. Since artifacts are always introduced to some extent, the determination of chemical concentrations in the pore water is recommended, in addition to the regular contaminant measurements conducted in the whole sediment, as a means of providing information on routes and levels of exposure, aiding in the interpretation of test results, and identifying sources of toxicity.
- The measurement of several porewater features, a number of which can act as confounding factors (e.g., salinity, alkalinity, pH, conductivity, DO, NH<sub>3</sub>, H<sub>2</sub>S, Eh), should be recorded shortly after porewater collection and after storage. This would help in interpreting test results, understanding the contribution of these factors to concordance/discordance between solid-phase and porewater test methods, and contributing to TIE procedures.
- From the statistical point of view, confounding factors are best accounted for by normalization in univariate tests and by simply including the confounding variables in a multivariate analysis.
- Some potential confounding factors (e.g., salinity) should be adjusted prior to testing to assure that test conditions are compatible with the needs of the test species.
- The use of a variety of test species was recommended, in order to enrich the database and help account for different modes of action and species sensitivity.
- The use of indigenous species is not recommended or suggested as important for the understanding of potential biological impacts as identified from the results of porewater toxicity tests. The use of water column organisms for porewater toxicity tests was considered scientifically appropriate.
- The sediment depth to be sampled for pore water should match the depth of interest for each particular survey.
- The need for appropriate reference sites was discussed and it was concluded that reference sites should have similar sediment (and therefore porewater) characteristics and be selected from a location near the study site, or at least in the same ecoregion. It was also suggested that if a suitable reference sediment cannot be found, the performance control could be used for the statistical comparison.
- It is also important to know the tolerance levels of the test species to major confounding factors.
- Regulatory aspects of the use of porewater toxicity tests included the need for prior determination of the purpose for the testing in a specific regulatory program and the need to determine what question(s) are being asked, ensuring that the question(s) are appropriate for the specific regulatory application. Porewater tests were considered suitable for

several types of frameworks, but unsuitable for others, e.g., as stand-alone pass/fail methods or as a substitute for a solid-phase test. This corroborates the findings that the 2 tests represent different routes of exposure and that the feeding mode of a test species can be of critical importance for the exposure to certain chemicals.

- The incorporation of porewater toxicity test results into empirically-derived SQGs might generate guidelines that are more predictive of porewater effects, better explain the results of porewater tests, and might be more protective if the porewater toxicity tests were to pick up a biological response to a chemical or class of chemicals that was missed by the solid-phase testing.
- Among the desirable attributes for porewater toxicity tests used in the SQT approach, the ability to identify causality seemed to take precedence over other aspects, although it was recognized that non-specific assays are useful exploratory tools to identify toxicity.

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