An integrated approach to the toxicity assessment of Irish marine sediments. Application of porewater Toxicity Identification Evaluation (TIE) to Irish marine sediments.

Ailbhe Macken [a, *], Michelle Giltrap [a, b], Barry Foley [c], Evin McGovern [b], Brendan McHugh [b], Maria Davoren [a]

[a]Radiation and Environmental Science Centre, Focas Institute, Dublin Institute of Technology, Kevin St., Dublin 8, Ireland.

[b]The Marine Institute, Rinville, Oranmore, Co. Galway, Ireland.

[c]School of Chemical and Pharmaceutical Sciences, Dublin Institute of Technology, Kevin St., Dublin 8, Ireland.

*Corresponding author. E-mail: Ailbhe.Macken@Astrazeneca.com

Abstract

An integrated approach to the ecotoxicological assessment of Irish marine sediments was carried out between 2004 and 2007. Phase I Toxicity Identification Evaluation (TIE) of sediment porewaters from two sites on the east coast of Ireland were conducted. Initial Tier I screening of three Irish sites identified the need for TIE after significant toxicity was observed with *Tisbe battagliai* and the Microtox® assay at two of the assayed sites (Alexandra Basin and Dunmore East). Porewaters classified as toxic were characterised using four manipulations, ethylenediaminetetraacetic acid (EDTA) chelation, sodium thiosulphate addition, C_{18} Solid Phase Extraction (SPE) and Cation Exchange (CE) SPE. Prior to initial testing, and TIE manipulations, all porewater samples were frozen at -20 ºC for several months until required. After initial Tier I testing Alexandra Basin porewater was classified as highly toxic by both assays while Dunmore East porewater only warranted a TIE with *T. battagliai*. Results of TIE manipulations for Alexandra Basin porewater and the Microtox® Basic test were inconclusive. The toxicity of the porewater in this assay was significantly reduced after freezing. Three experimental episodes were conducted with one month between each for the Alexandra Basin porewater. After each month of freezing the baseline toxicity was further reduced in the Microtox® assay, therefore it was not possible to draw accurate conclusions on the nature of the active contaminants in the sample. However, toxicity to *T. battalgiai* did not change after storage of the porewater. The C_{18} and CE SPE decreased the toxicity of Alexandra Basin porewater to the copepod indicating that both organic and cationic compounds (e.g. metals) were active in the sample. Dunmore East porewater was assayed with *T. battalgiai* and again a combination of organic and inorganic compounds were found to be partly responsible for the observed toxicity ($C_{18,}$ CE SPE and EDTA reduced toxicity).

Results from these TIEs provide insight into the complexity of interpreting marine TIE data from porewater studies where mixtures of unknown substances are present.

Key words: Sediment porewater; TIE; *Tisbe battagliai*; Microtox®

1 Introduction

Current risk assessment procedures within Europe rely solely on the correlation of toxicity and contaminant concentrations to suggest causes of observed effects. Although this method is used internationally it has many limitations, compounds causing the observed toxicity may not be included in the survey of chemicals, concentration of toxic chemicals may vary, it may be difficult to assess the bioavailability of contaminants measured in the sediment, and possible interactions (e.g. synergistic, antagonistic or additive effects) may not be taken into account. In the past two decades there has been a move towards an integration of chemical and biological methods to identify and characterise toxic components present in marine and freshwater sediments. When evaluating environmental samples the possible effects of mixtures are rarely considered. Therefore the use of methods such as Toxicity Identification Evaluation (TIE) allow for the examination of these possible effects.

Toxicity Identification Evaluation is a well-established technique originally developed by the US EPA (Mount and Anderson-Carnahan, 1988). Figure 1 summarises the history of TIE techniques and their use in the assessment of sediments. Unlike chemical analysis, which measures specific chemicals in a sample, TIE techniques allow for the identification of bioavailable fractions of chemicals, they address multiple toxicant interactions, and establish direct relationships between toxicity and analytical outputs. Despite their benefits these techniques are not widely employed in Europe (including Ireland) for regulatory purposes or risk assessment. Toxicity Identification Evaluation techniques involve three phases. Phase I involves characterizing the class or classes of contaminants contributing to the toxicity. Phase II involves the identification of specific toxicants that could be responsible and Phase

III is used to confirm that the toxicants identified in phase II are the cause of the toxicity observed in Phase I.

Although there are several methods for bulk sediment TIEs in the literature (Burgess et al., 2003; Ho et al., 2004), there are no standardised methods for whole sediment manipulations (Kwok et al., 2005). Toxicity Identification Evaluation methods to date have focused mainly on sediment interstitial water or porewater. Porewater is a major route of exposure for many water-soluble toxicants (Adams et al., 1992; Chapman et al., 2002). The use of an aqueous phase for the bioassay-directed fractionation eliminates the associated problems of whole sediment testing as many test organisms are incompatible with a solid matrix. For these reasons porewater was chosen for TIE assessment in this study.

When evaluating sediment quality the ideal scenario would be to measure *in situ* toxicity, however this is not always possible. When sampling sediment for the purpose of bioassay evaluation it is important to use methods of collection, handling and preservation which cause the least disturbance to the sediment geochemistry (Lamberson et al., 1992). Sediment bioassay samples need to be taken from the oxic fraction of surfical sediments (*i.e.* usually the top 5 cm). Information on storage of sediments in the literature varies greatly but the most highly advocated method is to store the sediment for no longer than 2 weeks at 4 ºC in the dark (ASTM, 1994). However this does not take into account the time involved in the extraction procedure (porewater, elutriate, solvent extract) and subsequent assessment with a battery (minimum of three bioassays representing different trophic levels and phyla) of tests in triplicate and further TIE manipulations (Phase I, II and III). Realistically and logistically it is not possible to conduct all of the described work within two weeks.

The effects of sediment storage (freezing and freeze drying) have been well studied and different authors have obtained varying results. Schuytema et al. (1989) found toxicity to decrease with storage time while Phelps and Warner (1990) and Dillon et al. (1994) observed an increase in toxicity with increased storage time. Geffard et al. (2004) assessed the effects of three storage methods (fresh, frozen and freeze-dried) and four storage periods (5, 15, 60 and 120 days). Geffard et al (2004) found that the effects of freezing and freeze-drying increased the toxicity of whole sediments and elutriates compared to fresh sediments no matter what type of contaminants were present in the sample (metals, polycyclic aromatic hydrocarbons (PAHs) or both). They also found that apart from elutriates extracted from sediments stored at 4 ºC, storage duration did not significantly alter elutriate toxicity test results with *Crassostrea gigas* embryos or larvae.

This study comprises part of an overall project on Irish marine sediment assessment. The first part of this study employed a multi-trophic battery of marine bioassays to assess the various phases of several sediments sampled from around Ireland (Macken et al., In Press). In the study all experiments were conducted on frozen samples that had been extracted (porewater, elutriates and solvent extracts) prior to freezing and no significant alteration in toxicity was observed during the assessment with all battery species (approx 3 – 4 weeks). Whole sediment testing was conducted immediately after sampling and was conducted on fresh sediment (i.e. within two weeks). However, for TIE manipulations the porewater was stored for approximately three months allowing the investigation of any alteration in toxicity with increased storage duration.

Due to its proximity to the bulk sediment, porewater is assumed to be similar in many of its characteristics to the surrounding sediment. Porewaters can act as a

pathway to contaminants that later become bound to the sediment. However some researchers have noticed that porewater, overlying water and sediment can be chemically different to each other (Nipper et al., 1998). Physio-chemical parameters such as pH, salinity, dissolved oxygen, sulphides and ammonia can vary greatly between porewater and the overlying water.

Confounding factors such as ammonia, sulphide and grain size are common issues in sediment toxicity assessment. It is important to take them into account as they may contribute to the observed toxicity but they themselves cannot be considered contaminants. Ammonia is an important confounding factor in sediment bioassays and needs to be taken into account. If a sediment sample has a high level of organic matter (> 2 ppm) and a large fraction of small sediment particles (< 63 µm), it is more likely to contain high levels of ammonia (Lapota et al., 2000). Conversely sediments with low levels of organic matter (< 0.5 ppm) and a larger sediment particle size (< 2mm) will contain low levels of total ammonia (Lapota et al, 2000). Ammonia is highly toxic to a variety of routinely employed aquatic bioassay species (Arizzi Novelli et al., 2003). Ammonia occurs naturally within sediments as a result of bacterial decomposition of organic matter (O'Neill, 1985). However, it is one of three classes of toxicants suspected of causing the majority of observed toxic sediment effects, the other two being organics and metals (Ankley et al., 1990; Ho et al., 2002). Zeolites have been used to reduce toxicity of ammonia in freshwater sediments (Besser et al., 1998), while both zeolites, *Ulva lactuca* and aeration methods have been used in marine whole sediment and porewater TIEs (Burgess et al., 2003).

Another well documented confounding factor in sediment toxicity testing is sulphide. Sulphide is produced by anaerobic decomposition of organic matter and can be an abundant constituent in marine sediments. Sulphides are considered more toxic

than ammonia under certain conditions (Lapota et al., 2000). The US EPA (2002) saltwater criterion for hydrogen sulphide is 2 µg l^{-1}, while for un-ionised ammonia the criterion is 35 µg l^{-1} (Lapota et al., 2000). As in the case of ammonia, sulphide production occurs in sediment with large amounts of organic matter and is pH dependent. Sulphide is volatile and oxidised and is, therefore, difficult to maintain at a constant concentration during toxicity testing. As a result no definitive dose-response relationship for sulphide threshold levels has been determined for benthic organisms. Low oxygen levels generally accompany increased sulphide concentrations which can in turn act as a confounding effect in sediment toxicity. It is important in sediment porewater (and bulk sediment) TIEs to take into account effects due to ammonia and sulphides as these compounds occur naturally (macrocompounds). As they do not only result from anthropogenic activities they cannot be described as "contaminants". In this study pH, dissolved oxygen and salinity were maintained at recommended guideline levels in order to reduce variations between experiments and diminish the possible effects of changing sulphide and ammonia levels in the porewater.

Initial toxicity test results from the multi-trophic battery of marine bioassays for all sites was used to judge how toxic the sediment samples were and if a TIE was warranted. Two sites, Dunmore East and Alexandra Basin, were identified as meriting a TIE investigation. The Microtox® and *T. battagliai* acute toxicity tests were selected as test organisms for the TIE as they were identified during previous research as being the most suitable for the sensitive, rapid testing of TIE manipulated porewater (Macken et al., In press). One of the main advantages of these tests is that they require very low volumes of test material, 2.5 ml and 45 ml for the Microtox® and *T. battagliai* tests respectively, compared to other bioassays (e.g. algal bioassays

requiring > 100 ml test solution, or oyster embryo-larval development assays requiring 200 ml test volume). As they are relatively inexpensive tests to conduct they allow for the assaying of many samples in tandem, at a low cost.

The aim of this study was to utilize TIE techniques to identify the bioavailable components causing the observed toxicity of the sediment samples previously identified (Macken et al., In Press) and investigate the effects of storage duration of frozen porewater samples. This paper describes four Phase I TIE methods used to identify the agents of toxicity and discusses the findings in relation to sediment chemistry. In the present study only Phase I techniques were applied and of these several manipulations were not employed (e.g. pH manipulation, *Ulva lactuca* ammonia removal and aeration). These manipulations were excluded as there was a limit on the amount of porewater that could be obtained from each site. Therefore a selection of manipulations encompassing as many classes of contaminants as environmentally significant (based on determined chemistry data for these sites) (*e.g.* metals and organics) as possible were incorporated into the experimental scheme.

2 Materials and Methods

2.1 Sample preparation

Alexandra Basin (AB) and Dunmore East (DE) sediments were sampled from onboard a small vessel and off the harbour wall respectively with a Van Veen Grab. Collected sediment was stored in polyethylene bags and transported back to the laboratory for storage at 4°C.

Before porewater extraction procedures, sediment was fully homogenised using a teflon spatula. Porewater extracts were prepared by centrifuging 25 - 40 ml sub-samples of the homogenised sediment at 1,200 x g for 30 min at 4 ^{o}C. The supernatant was collected as porewater and following filtration through a 0.2 µm filter (Nalgene, NY, USA), conductivity and salinity (Sension^{TM} electrode model number 51975-00), and pH (Sension^{TM} electrode model number 51935-00) were measured with a Sension^{TM} multimeter (Hach, Loveland, CO, USA). The sediment was processed within 2 – 4 days after collection and extracted porewater placed in amber glass bottles with minimal headspace and immediately frozen at -20 ºC and kept until required.

Prior to this study a battery of tests were previously used to evalute marine sediments from three sites around Ireland (Macken et al, In Press. In that study, the Microtox® solid phase test (SPT) and the 10-d acute amphipod test with *Corophium volutator* were used to assess whole sediment toxicity. Porewater and elutriates were assessed with the Microtox® acute test, the marine prasinophyte *Tetraselmis suecica*, and the marine copepod *Tisbe battagliai*. Solvent extracts were assayed with the Microtox® and *T. battagliai* acute tests.) were conducted prior to TIE manipulations on the identified toxic sites. Therefore the porewater was stored for up to three months after initial testing. A comprehensive chemical analysis was conducted on the

bulk sediment from both sites but no chemistry was performed on the porewater as there was an insufficient volume after manipulations from either site.

2.2 *Toxicity Identification Evaluation procedures*

Manipulations were carried out on the DE (*T. battagliai*) and AB (Microtox® and *T. battagliai*) porewaters. Four different marine porewater TIE manipulations were performed in accordance with methods described by Burgess et al., (1996). Porewater samples classified as toxic in the first part of this study (Macken et al., In Press), were further characterised using EDTA chelation, sodium thiosulphate addition, C_{18} Solid Phase Extraction (SPE) and cation exchange (CE) SPE (Figure 2). All TIE manipulations were conducted on porewater samples that had been stored at -20 ºC (three months). The aeration manipulation was omitted as the samples were adequately aerated after handling and had sufficient dissolved oxygen content for the test with *T. battagliai.* Due to the turbid nature of some of the extracted porewater samples a filtration step (passed through < 0.2 µm filter) was conducted on all samples prior to initial toxicity testing with all bioassays and prior to all TIE manipulations. However, to investigate the possible influence on toxicity by suspended particles an unfiltered sample of AB porewater (the most turbid sample) was tested with the Microtox® acute test and compared to the initial toxicity test (filtered sample).

2.3 *Porewater TIE testing with Vibrio fischeri (Microtox®)*

Due to the significant toxic effect observed at the top concentration with the EDTA and the 90 % Microtox® test, the Basic test (Azur Environmental, 1998) was employed for all spiking experiments.

For the Microtox® manipulations filtered porewater (100 %) samples were divided into five 5 ml portions. The first aliquot was used to measure the toxicity of the untreated samples. For the second and third aliquots 10 µl and 17 µl of ethylenediaminetetraacetic acid (EDTA) stock (74.68 mmol EDTA l^{-1}) and sodium thiosulphate stock (94.7 mmoles $Na_2S_2O_3$ l^{-1}) were added respectively and solutions were shaken. After addition of EDTA the sample was left to chelate for three hours prior to testing. In the case of the sodium thiosulphate the sample was left for one hour before testing. The fourth aliquot was passed through a methanol-activated C_{18} resin column (SUPELCO Discovery® DSC-18, USA). The post column sample eluate was then tested for toxicity. The final aliquot was passed through a methanol-activated CE SPE column (Supelclean™ LC-WCX SPE, USA) and the eluate was tested. Ethylenediaminetetraacetic acid and sodium thiosulphate at the same concentrations as used in the manipulations were added to Microtox® diluent and tested, acting as blanks. This ensured *V. fischeri* tolerance to the reagents at concentrations sufficient to alter toxicant effects. Microtox® diluent was passed through the SPE tubes and tested prior to TIE manipulations, thereby functioning as procedural blanks. All tests were performed in accordance with operational procedures from Azur Environmental Ltd. (Azur Environmental Ltd, 1998) and 5, 15 and 30 min readings were recorded. Three testing episodes were conducted with one month duration between each episode.

2.4 Porewater TIE testing with Tisbe battagliai

For *T. battagliai* manipulations the filtered porewater (100 %) sample was again divided into five aliquots each of 10 ml. The first aliquot was used to measure the toxicity of the untreated sample (dilution series of 20, 40, 60, 80 and 100 %

porewater). The second aliquot was used to prepare the same five step concentration dilution series (20, 40, 60, 80 and 100 % porewater). Ten millilitres of each concentration was prepared. Twenty microlitres of EDTA stock were added to each test concentration and after three hours *T. battagliai* were added. For the third aliquot, another identical dilution series was prepared, 34 µl of sodium thiosulphate stock were added to each test concentration and after one hour the organisms were added. The fourth aliquot was passed through a methanol-activated C_{18} resin (SUPELCO Discovery® DSC-18, USA) and this post column sample eluant was used to prepare the five concentration dilution series. Organisms were then added immediately. The final aliquot was passed through a methanol activated CE SPE column (Supelclean™ LC-WCX SPE, USA), collected and a five concentration dilution series prepared. Organisms were then added immediately. Blanks were prepared with filtered (0.2 µm) natural seawater and tested for all reagents and SPE tubes. All toxicity testing was conducted with slight modifications according to the ISO method (ISO/DIS 14669, 1997). Mortality was recorded after 24 and 48 hours.

Three sampling episodes per site were conducted. Successive experiments were conducted within approximately one month of each other. During the interim porewater samples were stored at -20 ºC in the dark in amber bottles.

2.5 Statistical Analysis

All manipulations were performed in triplicate (quadruplicate for *T. battagliai*) in three independent experiments. Data was expressed as arithmetic mean ± standard error of the mean (SEM). The acute toxicity data for the Microtox® assays was analysed using MicrotoxOmni® software (SDI Europe, Hampshire, UK). Toxicity data were fitted to a sigmoidal curve and the Hill model was used to calculate

Effective Concentration (EC) and Lethal Concentration (LC) values. This analysis was performed using REGTOX-EV6.xls (Èric Vindimian http://eric.vindimian.9online.fr/), a curve fitting macro for Microsoft® Excel. Statistical analyses was carried out using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. These analyses were performed using MINITAB® release 14 (MINITAB Inc. PA, USA). Statistical significance was accepted at $p \leq 0.05$. Percentage inhibition data generated by the MicrotoxOmni® software were Arcsin transformed prior to statistical analysis to improve normality and homogeneity of variances and reduce the influence of outliers. To confirm the precision of tests, the coefficient of variation (CV) was calculated for all controls.

3 Results

The results of the chemical analysis of metals and total organics for both sites have previously been reported (Macken et al., In Press) and some of the results are shown in Table 1. The physio-chemical properties of the two sediment sites differed in relation to particle size distribution. The < 63 µm particle fraction was greatest in the DE sample (62.50 %) compared to the AB sample (29.90 %) and the AB site had a greater percentage of water (64.65 %) than the DE site (54.65 %). All physio-chemical characteristics for both sites have also previously been reported (Macken et al., In Press)

Of the two sediment sites subjected to TIE both were characterised by PAH and metal contamination (Table 1) and both contained levels of TBT and DBT above the proposed SQG levels. However, AB contained much higher levels of PAHs than the DE site. The levels of contaminants at the sites were compared to ERL and ERM values (Table 1). The ERL – ERM values mentioned are based on the composition of sediments in which biological effects have been observed. ERM is defined as the median concentration (50th percentile) of a contaminant observed to have adverse biological effects in literature studies. A more protective indicator of contaminant concentrations is the ERL criterion, which is the 10th percentile concentration of a contaminant represented by studies demonstrating adverse biological effects in the literature. Ecological effects are not likely to occur at contaminant concentrations below the ERL criterion (Long et al., 1998). Several of the PAH levels from the AB site exceeded the Effects Range-Low (ERL) levels as defined by Long et al. (1995), however none of the levels of PAHs from the DE site were of higher concentration than the ERL levels (Table 1). In the case of AB the predicted toxicity of the metals in the sediment on marine organisms was moderate with contaminant levels between

the ERL and Effects Range-Moderate (ERM) levels according to Long et al. (1995). For the AB site 7 of the 10 metals analysed for had values higher than the ERL (Hg, As, Cd, Cu, Pb, Ni and Zn) two of these metals also occurred at higher concentrations than the ERM. Apart from As, Cu and Zn metal concentrations in DE sediment were well below ERL values.

After extraction of porewater, other parameters were recorded (pH, conductivity, temperature, salinity) these have all been previously reported in Macken et al. (In Press). All of the parameters were maintained within validity criteria levels throughout testing (ISO/DIS 14669, 1997; Azur Environment Ltd., 1998). The porewater from AB was yellow-brown in colour and cloudy with suspended particulate matter (SPM). Dunmore East porewater was also highly coloured and also contained suspended solids. Therefore, all initial porewater samples used in this study were filtered (0.2 µm filter) prior to TIE manipulations. To investigate the effects of filtration, unfiltered samples of AB porewater were assayed with the Microtox® system. This treatment had a detoxifying effect. This suggests that some of the toxicity in the initial sample was due to SPM. All further samples were filtered prior to baseline testing and all results of manipulations compared to the filtered baseline test to identify contaminants that were not associated with SPM.

Phase 1 TIE manipulations conducted on porewater and assayed with the Microtox® system were highly variable. As previously mentioned tests were conducted on three independent days with approximately one month between each. After each set of manipulations the results were not reproducible. In the baseline toxicity test on Day 1 (first testing episode, within five days of freezing) the percentage inhibition of light was 90.72 % at the top concentration (45 % porewater). On Day 2 (one month after freezing) the light inhibition at the top concentration had

dropped to 65.00 % and on Day 3 (two months after freezing) the light inhibition at the top concentration was only 45.57 %. Therefore any subsequent manipulations were only comparable to the baseline on that day. No conclusions can be made as the experimental manipulations were not reproducible following the various periods of freezing.

Results of manipulated DE porewater assayed with *T. battagliai* are shown in Figure 3. A significant reduction in porewater toxicity compared to the baseline only occurred after 48 h at 40 % porewater after elution through the CE SPE column. However, if one were to compare calculated EC_{50} values (Table 2), reductions in toxicity occurred after 48 h with the EDTA, C_{18} and CE SPE manipulations. According to EC_{50} values the most effective method of reducing toxicity in the sample was the CE elution. Sodium thiosulphate was observed to increase the toxicity of the porewater after both 24 and 48 h. Based on these results it can be concluded that both organic compounds and cationic compounds (e.g. metals) were active in the original sample. None of the manipulations employed in this study completely removed the toxicity of the sample to *T. battagliai* therefore it can be assumed that there were other factors active in contributing to the observed toxicity.

Results of TIE manipulations with the AB porewater and *T. battalgliai* are shown in Figure 4. Addition of sodium thiosulphate increased the toxicity of the porewater (Table 2) after 24 h, however, this effect was only significant at 20 % porewater (Figure 4). The addition of EDTA significantly reduced the toxicity at 20 % (24, 48 h) and 40 % (48 h) porewater. Passage through both the C_{18} and CE SPE columns removed the toxicity significantly at all concentrations from 20 – 80 % after both 24 and 48 h exposure (Figure 4). The corresponding EC_{50} values also show a considerable reduction in toxicity after both SPE manipulations (Table 2). Unlike

the Microtox® assay only a slight reduction in baseline toxicity after periods of freezing occurred with the *T. battagliai* assay.

4 Discussion

Due to the associated problems with obtaining porewater, test species that use small sample volumes are most amenable for testing with sediment porewater especially in TIE studies where multiple manipulations are required. Hence, *T. battagliai* and the Microtox® system were employed in this study. Porewater was selected for use in the TIE manipulation as it is considered to contain the most bioavailable fraction of contaminants (Carr, 1997; Nipper et al., 2002). This study forms part of an integrated project on marine sediment assessment. The use of these techniques, which have not previously been employed in an Irish environment, allow us to investigate the effects of mixtures of contaminants, identify possible agents of toxicity and include sediment chemistry to support any noteworthy observations.

In general it is almost impossible to extract porewater and expose test organisms to the sample without altering the chemistry of the sample and its natural, anthropogenic and organic components. A Society of Toxicology and Chemistry (SETAC) technical workshop on porewater toxicity testing outlined recommendations in their summary report on the effects of sampling, storage, handling and toxicity testing of porewater (Adams et al., 2001). The report highlighted the problems associated with scheduling and other considerations which prevent toxicity tests being conducted immediately after sampling. If testing cannot be conducted directly after sampling, methods of storage and handling become important concerns. The SETAC working group recommended two options on how to minimise the effects: (1) storage of porewater *in situ* (i.e. store the sediment sample as intact as possible), while maintaining it at 4 ºC to slow biological/biochemical processes, then porewater should be extracted immediately before assaying; or (2) extraction of the porewater and storage at 4 ºC (short-term) or frozen (long-term). The key to working with porewater

and minimising any effects on geochemistry is to keep storage time to a minimum where possible. However, this is not always possible. As this study was conducted over several years and was part of a larger project, the scheduling of experiments did not allow for immediate TIE analysis following Tier 1 multitrophic battery assessment. Porewater was extracted within 2 – 4 days of sediment sampling and immediately frozen. Toxicity testing of baseline and TIE manipulated porewater with the Microtox® and *T. battagliai* system was conducted on three separate occasions with one month duration between each testing episode. At each testing point replication was employed in triplicate (Microtox®) and quadruplicate (*T. battalgiai*).

Initial toxicity testing with AB porewater resulted in EC_{50} values of 7.44, 7.71 and 11.34 % for 5, 15 and 30 min respectively with the Microtox® system. While initial testing with *T. battagliai* resulted in much higher 24 and 48 h LC_{50} values of 32.42 and 24.59 % (previously reported data Macken et al. In Press). These results imply that the Microtox® system was more sensitive to the constituents in the original sample of AB porewater. After storage there was little change in the toxicity of the porewater to *T. battagliai*, however, the toxicity to the Microtox® was found to decrease.

It was demonstrated that storage duration of frozen porewater decreased the toxicity of the sample to *V. fischeri* (Microtox®) therefore the causative agents of toxicity in the original sample could not be identified. Schuytema et al., (1989) observed a similar decrease in the bioavailability and toxicity of organic compounds contained in sediments after freezing. As previously mentioned, AB sediment was characterized by high PAH contamination (values of several analysed PAHs exceeded the ERL values [Long et al., 1995]) and if these constituents were present in the porewater they could have been altered during storage.

The results with *T. battagliai* for both the AB and DE porewaters were more reproducible. Fifty percent mortality after baseline toxicity testing with *T. battagliai* and DE porewater was observed at 45.81 % (24 h) and 32.11 % (48 h). Therefore there was a slight reduction in toxicity of the sample between initial and baseline testing (Table 2). After storage and baseline testing the toxicity to *T. battagliai* was not found to significantly decrease as in the Microtox® assay. These results hint that the toxicants in the AB porewater were acting differently on the two test organisms and that the causative agents of toxicity to the two test species were affected differently by the storage method and duration. Several studies have shown that freezing has a minimal effect on the toxicity of sediment (Carr and Chapman, 1995; Norton et al., 1999), therefore freezing was employed in this study considering the time constraints. Although the main aim of this study was to conduct TIE on porewaters classified as toxic following initial Tier 1 assessment the results showed that freezing had a significant effect on toxicity, therefore, highlighting the influence of storage methods and duration for certain assays (e.g. Microtox®).

The results of the porewater TIE indicate that both DE and AB contained a mixture of organic and divalent cationic contaminants which contributed to the toxicity observed in the initial battery assessment. The addition of sodium thiosulphate increased the toxicity of the porewater from both sites to *T. battagliai*. Blanks of all reagents were run with all manipulations and no significant toxicity was observed with sodium thiosulphate. A previous study (Macken et al., 2008) with *T. battalgiai* and the TIE reagent sodium thiosulphate has shown that addition of this reagent to a sample containing low levels of TBT can increase the sample toxicity. Bulk sediment chemistry data shows the presence of the organotin compound TBT at both sites (Table 1), therefore this may explain the increase in toxicity, compared to

the baseline, at low levels of porewater. However, it should be noted that these contaminants were not responsible for the total toxicity as none of the employed manipulations completely removed the toxicity of the sample. As previously outlined confounding factors such as ammonia and sulphides as well as changes in physio-chemical parameters (e.g. pH, temperature, dissolved oxygen) can contribute to the toxicity of sediments both in their bulk and aqueous phases.

Ho et al. (2002) stated that there is no predominant cause of toxicity in sediments where ammonia, metals and organics all play a fairly equal causative role. However, marine sediments are considered an exception; in their case, metal toxicity was considered a minor factor. Hansen et al. (1996) thought that these effects may be the result of higher concentrations of sulphides (another confounding factor) found in marine sediments, which bind to many toxic metals reducing their toxicity. However, De Lange et al. (2008) found that storage conditions increased the levels of acid volatile sulphide (AVS) but did not effect the concentrations of simultaneously extracted metals (SEM). Sulphites were not included in the suite of chemicals analysed for in this study as samples for chemical analysis were subjected to a sulphur clean up prior to testing. Ho et al. (2002) also indicated that in recent years ammonia has been found to play a much larger role in sediment toxicity than was previously expected.

As ammonia toxicity was not evaluated in this study it may have been responsible for some of the observed toxicity at both sites and this may have been influenced by the duration of storage prior to assessment. It was not possible to conduct further tests to remove ammonia or conduct graduate pH procedures as there was a limit on the amount of porewater that could be extracted from the original samples. Losso et al. (2007) concluded that the high toxicity observed in their

elutriate samples could not be attributed to ammonia toxicity alone and that only a few samples had ammonia concentrations similar to the NOEC value for their test species (*Paracentrotus lividis* and *C. gigas*) or higher, however, some samples did exceed the sensitivity limit of the methods. In their study, elutriates were stored at -18 ºC prior to toxicological analysis for an undisclosed duration. Chien et al. (1990) found that freezing and freeze-drying of sediments led to an increase in ammonia concentrations in elutriates. Geffard et al. (2004) also determined that the duration of storage time of fresh, frozen and freeze-dried sediment increased the levels of ammonia to above the NOEC level for *C. gigas*. Although levels of ammonia in frozen and freeze-dried sediments were greater than the fresh sediment after short-term storage (5 days), they faired much better than the fresh sediment after long-term storage (120 days). In our study the toxicity was observed to decrease with the duration of storage time and hence it is unlikely an increase in ammonia was responsible for all the remaining porewater toxicity.

Unfortunately it was not possible to conduct chemical analysis (for metals and organic compounds) on the porewater so only whole sediment chemistry was available for comparative purposes. Geffard et al. (2004) found that from a concentration point of view the rank of elutriates in relation to chemistry data was similar to the rank obtained with whole sediment. However, we cannot be sure about the levels of contaminants that were dissolved in the porewater. Carr (1997) stated that although different porewater extraction methods yield samples with similar toxicity certain types of contaminants may be preferentially lost (e.g. volatile organics) or concentrated (e.g. oil and PCBs) by centrifugation or lost through adsorption (e.g. volatile organics) during filtration (filtration of all porewater samples was conducted prior to testing in this study). The same method of extraction was used for all

porewaters to minimise the influence of this variable among samples (Macken et al. In Press).

There are many advantage of using porewater for toxicity assessment and TIE. Unlike elutriates the porewater is in direct contact with the sediment fraction and is therefore a more relevant phase for investigation. As previously mentioned, this study was part of a larger integrated project and further work on the bioassay directed fractionation of organic solvent extracts and the DE sediment was also conducted (manuscript in preparation). However, the use of porewater TIE is less manipulative than solvent extracts and there are no residual solvent concerns. Porewater testing and TIE also allow for the assessment of dissolved phase associated sediment contaminants (e.g. route-of-exposure information). Therefore the use of porewater for TIE manipulations is advocated where whole sediment TIE is not possible.

Further modifications in regard to TIE methodologies for porewater are required in several aspects. The need to determine acceptable sediment/porewater storage time is a must as is the further investigation of effects of porewater extraction and storage on microbial degradation of contaminant and ammonia production. In all TIE studies there is a need to assess the threshold levels of confounding factors (e.g. ammonia, sulphides, pH) for all employed species. Even in this study where the samples volumes required were low there was insufficient porewater extracted to allow for a full TIE and further chemical analysis. Therefore there is a need to develop a broader range of short-term acute and chronic test which require small volumes of porewater TIE (e.g. algal microplate method). From this limited study (two species employed for TIE) the importance of utilizing several trophic level representatives is apparent as it is clear that the causative agents of toxicity were not the same for each species. Therefore the use of one single assay for TIE is

discouraged and the employment of a battery approach is strongly advocated where possible.

In conclusion, the Phase I toxicity characterisation of both porewater samples with *T. battagliai* found the C_{18} and CE SPE column manipulations to be the most successful in reducing toxicity of the samples. These results suggest that some of suspected toxicant(s) are cationic divalent metals or organic compounds. The filtration step significantly reduced toxicity of porewater in the Microtox® system implying that particulate bound contaminants may also have contributed to the toxicity of the AB sample *in situ*. No TIE manipulation used in this study removed all of the toxicity at either site. Therefore there were other contaminants active in the sample that contributed to the toxicity. These may have been macromolecules such as sulphides and ammonia which are frequently present in marine sediments at elevated levels and may be responsible for the observed toxicity. The effects of storage on the reduction of toxicity in the Microtox® system also emphasizes the importance of the treatment of sediment after sampling to reduce the appearance of artefacts which may influence or alter the sample toxicity giving an inaccurate assessment of the state of the environment. It is suggested that future studies incorporate more rigorous chemical analysis of sediment phases for direct comparison with TIE results and that more species be included to account for varying sensitivities.

In regard to the issue of storage method and duration it is recommended that sediment be stored intact at 4 ºC and porewater be extracted only prior to use if testing can reasonably be completed within two weeks. All toxicity testing should be conducted as soon as possible after collection but the associated difficulties when dealing with a large battery of tests with large sample volumes needs to be highlighted (time involved to extract sufficient quantities for assays such as traditional algal flask

assays). From results of studies in the literature as well as our own findings it is obvious that the effects of storage are not the same for all sediments. We can therefore surmise that freezing and duration of storage has a very different effect on different contaminants and sediment types (i.e. levels of total organic carbon, grain size etc.) as well as the toxicity to different organisms. In cases where testing cannot reasonably be conducted within two weeks it is recommend that porewater be extracted as soon after sampling as possible and immediately frozen. However, we advise that the storage duration (freezing) should be kept to a minimum where possible.

Acknowledgements

We sincerely wish to thank the Shannon Aquatic Toxicological Laboratory (SATL), especially Robert Hernan and Kathleen O'Rourke, for supplying the *Tisbe battagliai* and providing technical assistance for starting up our own culture facility.

Funding for this research was provided by Technology Sector Research: Strand III: Core Research Strengths.

References

Adams, E.J.; Kimerl, R.A.; Barrett, J.W. Sediment quality and aquatic life assessment. Environ Sci Technol. 26: 1864-1875; 1992.

Adams, W.J.; Berry, W.J.; Burton Jr.; G.A., Ho, K.; MacDonald, D.; Scroggins, R.; Winger, P.V. 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. 18-22 March 2000; Pensacola, FL. A SETAC Publication. pp. 38; 2001.

Ankley, G.T.; Katko, A.; Arthur, J.W. Identification of ammonia as an important sediment-associated toxicant in the Lower Fox River and Green Bay, Wisconsin. Environ Toxicol Chem. 9:313-322; 1990.

Ankley, G.T.; Schubauer-Berigan, M.K. Comparison of techniques for the isolation of sediment pore water for toxicity testing. Arch Environ. Contam Toxicol. 27: 507–512; 1994.

Ankley, G.T.; Schubauer-Berigan, M.K.; Dierkes, J.R. Application of toxicity identification evluation techniques to pore water from Buffalo River sediments. J Great Lakes Res. 22(3):534-544; 1996.

Arizzi Novelli, A.; Picone, M.; Losso, C.; Volpi Ghirardini, A. Ammonia as confounding factor in toxicity tests with the sea urchin *Paracentrotus lividus* (Lmk). Toxicol Environ Chem. 85(4-6):183-191; 2003.

ASTM (American Society for Testing and Materials). Standard guide for collection, storage, characterization and manipulation of sediment for toxicological testing, E1391-1394, Philadelphia, PA; 1994.

Azur Environmental Ltd. Microtox® acute toxicity basic test procedures. Carlsbad, CA, USA. 1998.

Besser, J.M.; Ingersoll, C.G. Leonard, E.N. Mount, D.R. Effect of zeolite on toxicity of ammonia in freshwater sediments: implication for toxicity identification evaluation procedures. Environl Toxicol Chem. 17(11):2310-2317; 1998.

Burgess, R.M.; Ho, K.T.; Morrison, G.E.; Chapman, G.; Denton, D.L. Marine toxicity identification evaluation (TIE). Phase I guidance document, EPA/600/R-96/054. Washington, DC, USA: Environmental Protection Agency. pp. 70; 1996.

Burgess, R.M.; Pelletier, M.C.; Ho, K.T.; Serbst, J.R.; Ryba, S.A.; Kuhn, A.; Perron, M.M.; Raczelowski, P.; Cantwell, M.G. Removal of ammonia toxicity in marine sediment TIEs: a comparison of *Ulva lactuca*, zeolite and aeration methods. Mar Pollut Bull. 46:607-618; 2003.

Carr, S. Marine and estuarine porewater toxicity testing. In: Microscale techniques in aquatic toxicology: Advances, techniques, and practice. P. G. Wells, Lee, K., Blaise, C. (Eds.). Boca Raton, Florida, CRC. pp. 650; 1997.

Carr, R.S.; Chapman, D.C. Comparison of methods for conducting marine and estuarine sediment porewater toxicity tests: Extraction, storage, and handling techniques. Arc Environ Contam Toxicol. 28: 69-77; 1995.

Chapman, P.M.; Wang, F.; Germano, J.D.; Batley, G. Pore water testing and analysis: the good, the bad, and the ugly. Mar Pollut Bull. 44: 359-366; 2002.

Chien, Y.H.; Chen, S.S.; Chen, C.G. Effects of freeze storage and centrifugal force on inorganic nitrogen content in interstitial water of submerged sediment. J. Fish Soc Taiwan. 17(3): 199-206; 1990.

De Lange, H.J.; Van Griethuysen, C.; Koelmans, A.A. Sampling method, storage and pretreatment of sediment affect AVS concentrations with consequences for bioassay responses. Environ Pollut. 151: 243-251; 2008.

Dillon, T.M.; Moore, D.W.; Jarvis, S.A. The effects of storage temperature and time on sediment toxicity. Arch Environ Con Tox. 27:51-53; 1994.

Geffard, O.; His, E.; Budzinski, H.; Chiffoleau, J.F.; Coynel, A.; Etcheber, H. Effects of storage method and duration on the toxicity of marine sediments to embryos of *Crassostrea gigas* oysters. Environ Pollut. 129: 457-465; 2004.

Guzzella, L.; Bartone, C.; Ross, P.; Tartari, G.; Muntau, H. Toxicity identification evaluation of Lake Orta (Northern Italy) sediments using microtox system. Ecotox Environ Saf. 35:231-235; 1996.

Hansen, D.J.; Berry, W.K.; Mahony, J.D.; Boothman, W.S.; Di Toro, D.M.; Robson, D.L.; Ankley, G.T.; Ma, D.; Yan, Q.; Pesch, C.E. Predicting the toxicity of metal-contaminated field sediments using interstitial concentrations of metals and acid-volatile sulphide normalizations. Environ Toxicol Chem. 15: 2080-2094; 1996.

Ho, K.T.; McKinney, R.A.; Kuhn, A.; Pelletier, M.C.; Burgess, R.M. Identification of acute toxicants in New Bedford Harbour sediments. Environ Toxicol Chem. 16:551-558. 1997.

Ho, K.T.; Kuhn, A.; Pelletier, M.C.; Serbst, J.R.; Ryba, S.A.; Cantwell, M.G., Kuhn, A.; Raczelowski, P. An overview of toxicant identification in sediment and dredged material. Mar Pollut Bull. 44:286-293; 2002.

Ho, K.T.; Burgess, R.M.; Pelletier, M.C.; Serbst, J.R.; Cook, H.; Cantwell, M.G.; Ryba, S.A.; Perron, M.M.; Lebo, J.; Huckins, J.; Petty, J. Use of powdered coconut charcoal as a toxicity identification and evaluation mainpulation for organic toxicants in marine sediments. Environ Toxicol Chem. 23:2124-2131; 2004.

ISO/DIS 14669 (International Organization for Standardization). Water Quality - Determination of acute lethal toxicity to marine copepoda (Copepoda, Crustacea), International Standard ISO/DIS, Geneva, Switzerland; 1997.

Kosian, P.A.; Makynen, E.A.; Monson, P.D.; Mount, D.R.; Spacie, A.; Mekenyan, O.G.; Ankley, G.T. Application of toxcity based fractionation techniques and structure activity relationship models for the identification of phototoxic polycyclic aromatic hydrocarbons in sediment pore water. Environ Toxicol Chem. 17:1021-1033; 1998.

Kwok, Y.C.; Hsieh, D.P.H.; Wong, P.K. Toxicity evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Mar Pollut Bull. 51(8-12): 1085-1091; 2005.

Lamberson, J.O.; Dewitt, T.H.; Swartz, R.C. Assessment of sediment toxicity to marine benthos. In: Sediment Toxicity Assessment. Burton Jr., G.A. (Ed.). Lewis, Boca Raton, USA, pp. 183-211; 1992.

Lapota D.; Duckworth, D.; Word, J. Confounding factors in sediment toxicology. Issue papers:1-19. SPAWAR Systems Centre San Diego: 2000. http://web.ead.anl.gov/ecorisk/issue/pdf/confound.pdf.

Long, E.R.; MacDonald, D.D.; Smith, S.L.; Calder, F.D. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manage. 19: 81-97; 1995.

Losso, C.; Arizzi Novelli, A.; Picone, M.; Marchetto, D.; Pantani, C.; Ghetti, P, F.; Volpi Ghirardini, A. Potential role of sulphide and ammonia as confounding factors in elutriate toxicity bioassays with early life stages of sea urchins and bivalves. Ecotox Environ Saf. 66: 252-257; 2007.

Macken, A.L.; Giltrap, M., Foley, B., McGovern, E., McHugh, B., Davoren, M. An integrated approach to the toxicity assessment of Irish marine sediments. Validation of established marine bioassays for the monitoring of Irish marine sediments: Environ Inter. <DOI: 10.1016/j.envint.2008.03.005>. In Press.

Macken, A.L.; Giltrap, M., Foley, B., McGovern, E., McHugh, B., Davoren, M. A Model Compound Study: The ecotoxicological evaluation of five organic contaminants employing a battery of marine bioassays. Environ Pollut. 153(3): 627-637; 2008.

Mount, D.I.; Anderson-Carnahan L. Methods for aquatic toxicity identification evaluations. Phase I toxicity characterization procedures. EPA 600/3-88-034. U.S. Environmental Protection Agency, Duluth, MN; 1988.

Nipper, M.G.; Roper, D.S.; Williams, E.K.; Martin, M.L. Sediment toxicity and benthic communities in mildly contaminated mudflats. Environ Toxicol Chem. 17:502-510; 1998.

Nipper, M.; Carr, R.S., Biedenbach, J.M.; Hooten, R.L.; Miller, K. Toxicological and chemical assessment of ordnance compunds in marine sediments and porewaters. Mar Pollut Bull. 44: 789-806; 2002.

Norton, B.L.; Lewis, M.A.; Mayer, F.L. Storage duration and temperature and the acute toxicities of estuarine sediments to *Mysidopsis bahia* and *Leptocheirus plumulosus.* Bull Environ Contam Toxicol. 63: 157-166; 1999.

O'Neill P. Environmental Chemistry. Boston, MA: George Allen and Unwin. pp 192; 1985.

Phelps, H.L.; Warner, K.A. Estuarine sediment bioassay with oyster pediveliger larvae (*Crassostrea gigas*). Bull Envion Contam Toxicol. 44(2): 197–204; 1990.

Ross, P.; Dillon, F.S. Toxicity Identification Evaluation (TIE) with aquatic bottom sediments: A case study. In: Risk Reduction. M.L. Richardson (Ed.). Taylor and Francis.USA: 1996

Schuytema, G.S.; Nebeker, A.V.; Grifis, W.L.; Miller, C.E. Effects of freezing on toxicity of sediments contaminated with DDT and Endrin. Environ Toxicol Chem. 8: 883-891; 1989.

US EPA. (U.S. Environmental Protection Agency). Burgess, R.M.; Ho, K.T.; Morrison, G.E.; Chapman, G.; Denton, D.L. Marine toxicity identification evaluation

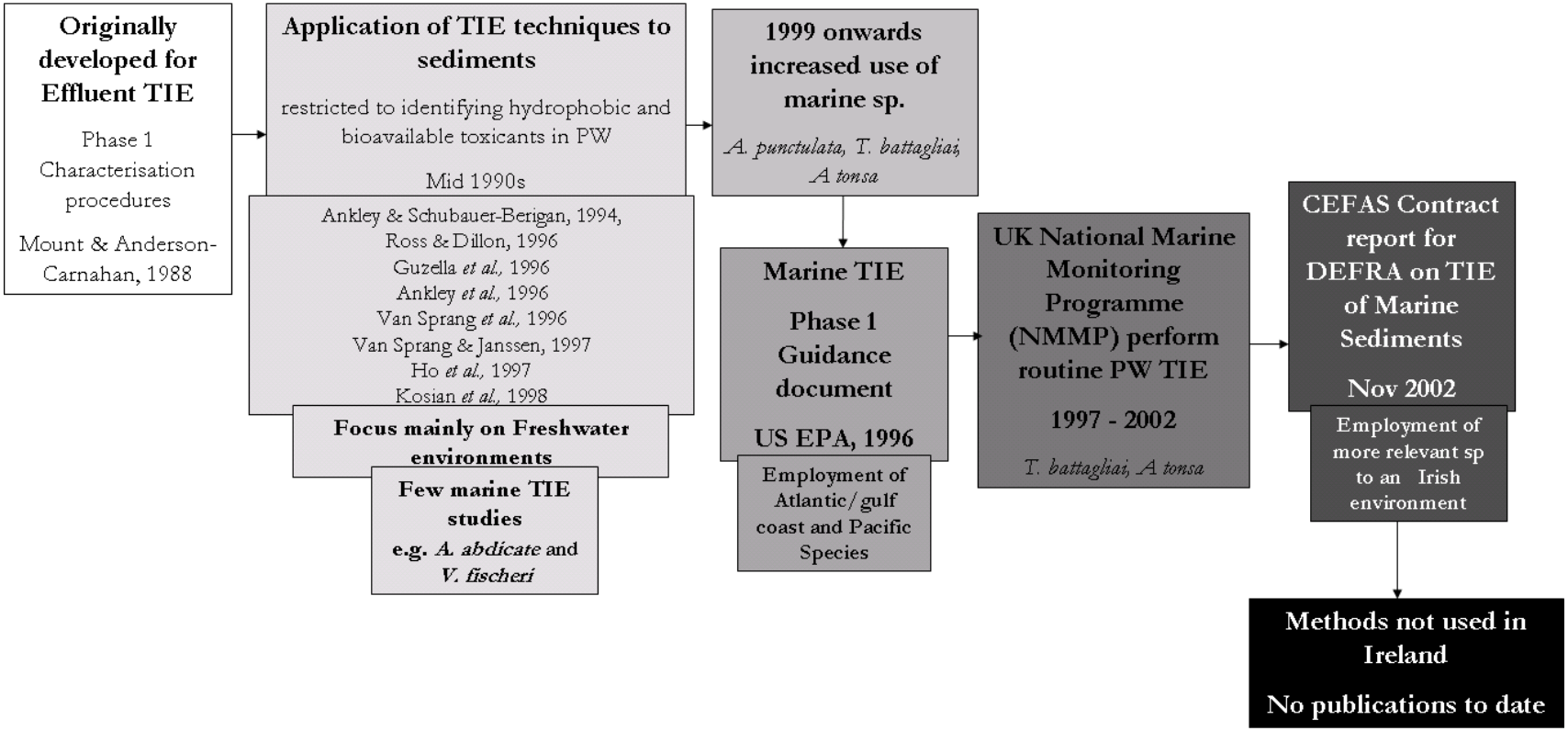
(TIE). Phase I guidance document, EPA/600/R-96/054. Washington, DC, USA: Environmental Protection Agency. pp. 70; 1996.

US EPA. (U.S. Environmental Protection Agency). National recommended water quality criteria: 2002. (EPA-822-R-02-047): pp 36; 2002.

Van Sprang, P.A.; Janssen, C.R.; Sabayasachi, M.; Benijts, F.; Persoon, G. Assessment of ammonia toxicity in contaminated sediments of the upper Scheldt (Belgium): The development and application of toxicity identification evaluation procedures. Chemosphere 33: 1967-1974; 1996.

Van Sprang, P.A.; Janssen, C.R. Identification and confirmation of ammonia toxicity in contaminated sediments using a modified toxicity identification evaluation approach. Environ Toxicol Chem. 16(12): 2501-2507; 1997.

1 **Figures and Tables**



2
3 Figure 1. Summary Flow diagram of the history of Toxicity Identification Evaluation as a technique for sediment evaluation.

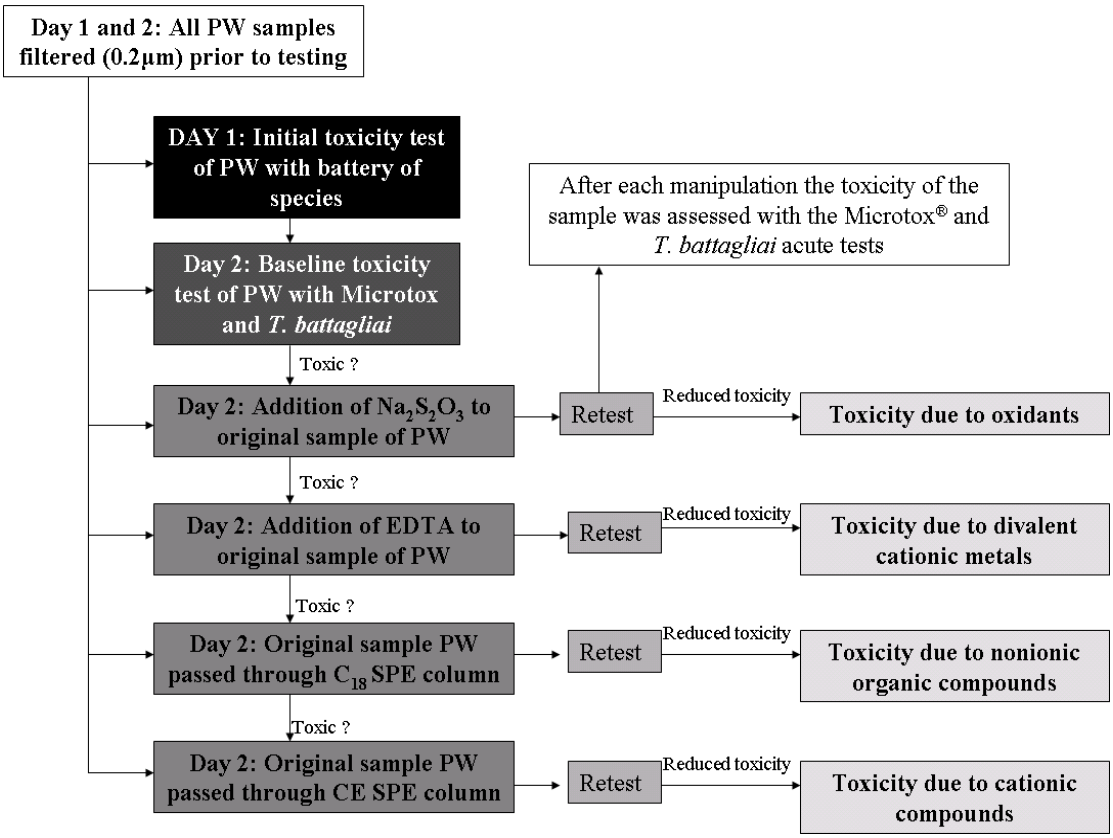
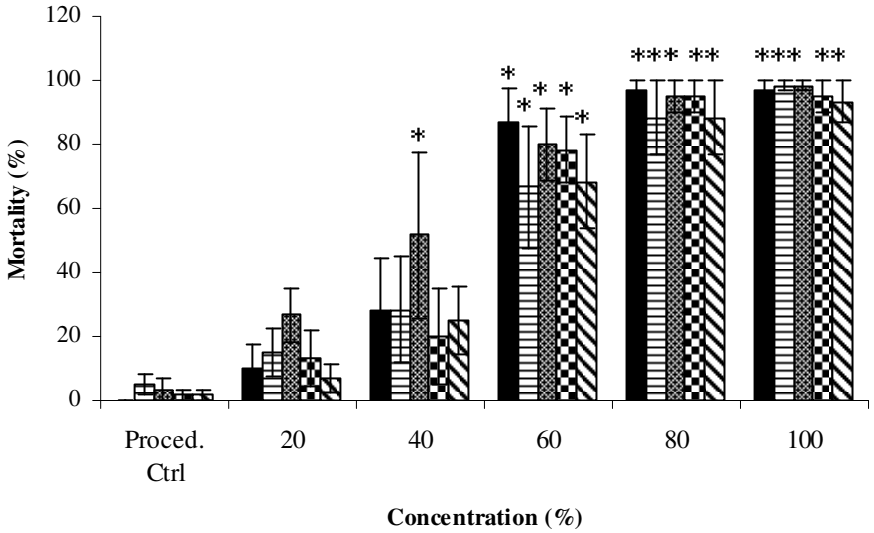


Figure 2. Overview flowchart for Toxicity Identification Evaluation procedure of marine porewater. Initial toxicity testing was conducted during battery testing (Day 1) while baseline toxicity testing and subsequent TIE manipulations were conducted on Day 2 (any time after initial battery testing). After each manipulation the resulting solution was tested with both the Microtox® and *T. battagliai* acute tests to identify any changes in toxicity post manipulation. PW = Porewater, EDTA = ethylenediaminetetraacetic acid, C_{18} SPE = Carbon 18 Solid Phase Extraction, CE SPE = Cation Exchange Solid Phase Extraction.

(a)



(b)

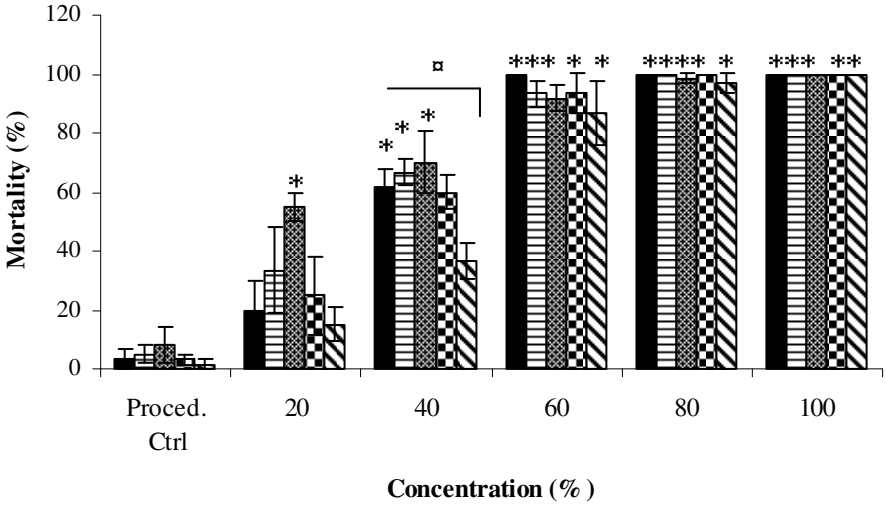
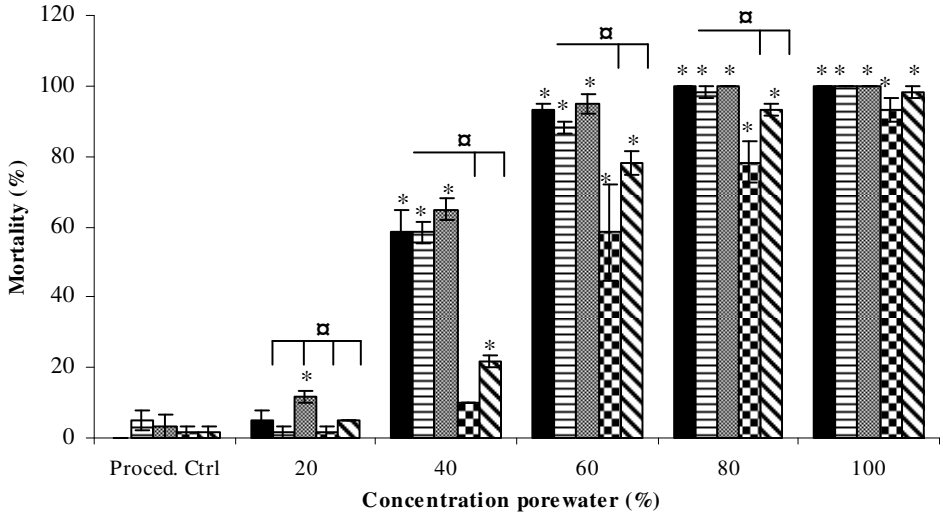


Figure 3. Percentage mortality of *T. battagliai* exposure to TIE manipulated porewater (Dunmore East) samples after Baseline testing (■), post EDTA addition (▤), post $Na_2S_2O_3$ addition (▩), post C_{18} (▦), and post CE SPE (▧) manipulation after (a) 24, and (b) 48 h exposure. Data are expressed as a percentage of unexposed controls ± SEM of three independent experiments. * denotes significance from the control ($p \leq 0.05$). ¤ denotes significant difference of each manipulation compared to baseline values at each concentration ($p \leq 0.05$). CV for the controls ranged from 0 -22.2 %.

(a)



(b)

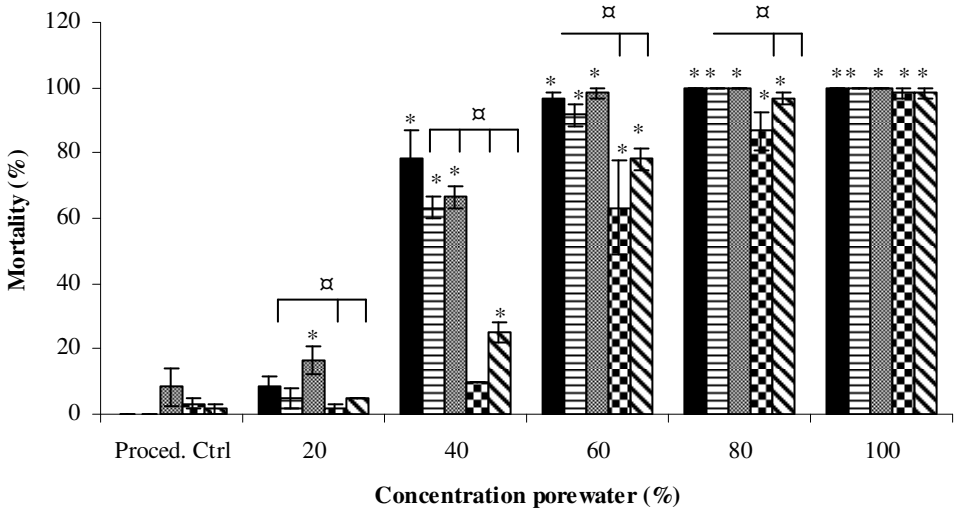


Figure 4. Percentage mortality of *T. battagliai* exposure to TIE manipulated porewater (Alexandra Basin) samples after Baseline testing (■), post EDTA addition (▤), post $Na_2S_2O_3$ addition (▩), post C_{18} (▦), and post CE SPE (▧) manipulation after (a) 24, and (b) 48 h exposure. Data are expressed as a percentage of unexposed controls ± SEM of three independent experiments. * denotes significance from the control ($p \leq 0.05$). ¤ denotes significant difference of each manipulation compared to baseline values at each concentration ($p \leq 0.05$). The CV for the controls was 0 %.

Table 1 Comparison of some ERL and ERM values (Long et al., 1995) with results of chemical analysis for metals and organics of the fine (< 2 mm) sediment fraction for Dunmore East and Alexandra Basin sites.

Chemical[a]	**Dunmore East**	**Alexandra Basin**	**ERL**[b]	**ERM**[b]
Metals (mg kg^{-1})				
Mercury	<0.05	0.283	0.15	0.71
Aluminium *(% dry weight)*	12800	19200	-	-
Lithium *(% dry weight)*	29.4	33.7	-	-
Arsenic	7.05	11.7	8.2	70
Cadmium	0.481	3.23	1.2	9.6
Chromium	41.7	41.7	81	370
Copper	76.8	78.8	34	270
Lead	45.4	265.0	46.7	218
Nickel	18.6	28.4	20.9	51.6
Zinc	242.0	755.0	150	410
Organic Contaminants (µg kg^{-1})				
Anthracene	29.4	168	85.3	1100
Fluoranthene	117	561	600	5100
Pyrene	153	617	665	2600
Benz[a]anthracene	63.6	382	430	1600
Benzo[a]pyrene	< 10	432	261	1600
Benzo[ghi]perylene	64.1	374	720[‡]	2600[‡]
Acenaphthene	< 10	75.1	16	500
Chrysene	129	435	384	2800
Benzo(k)fluoranthene	< 43.5[*]	202	280	1620
Benzo(b)fluoranthene	88.6	431	320	1880
Naphthalene	<43.1[*]	< 192[*]	160	2100
Fluorene	30	173	19	540
DDE *op*	< 3	< 3	1.6	27
DDE *pp*	< 3	< 3[*]	2.2	27
DDT *pp*	< 22.5	< 18.5[*]	1.6	27
PCB 028	< 3	<3.6[*]	22.7	180
PCB 052	< 3	<3.5[*]	22.7	180
PCB 101	< 3	4	22.7	180
PCB 138	4.7	4.3	22.7	180
PCB 153	< 3	< 3	22.7	180
PCB 180	< 3	< 3	22.7	180
PCB 118	3.4	3.4	22.7	180
TBT	2125	6621.42	100	500
DBT	790	1362.39		
MBT	609	683.48		

[a] Only chemistry data where there are corresponding ERL and ERM values are shown.
[b] ERL and ERM from Long, 1995
[‡] Apparent effects threshold – low/high
[*] Raised LOD due to ion interference
[§] Proposed lower and upper limits of Σ TBT & DBT

Table 2. Effect concentrations (EC_{10}, EC_{50}, NOEC, and LOEC) based on the *T. battagliai* acute toxicity tests completed using TIE manipulations of Dunmore East and Alexandra Basin porewater.

Site	Manipulation	Exposure time (h)	EC_{10}[a] (% PW)	EC_{50}[a] (% PW)	NOEC[b] (% PW)	LOEC[c] (% PW)
Dunmore East	Initial	24	16.67 (8.90 – 36.38)	32.65 (23.80 -42.04)	40	60
		48	8.65 (2.58 – 21.89)	22.21 (12.70 – 33.59)	40	60
	Baseline	24	32.82 (25.82 – 38.78)	45.81 (40.70 – 50.48)	40	60
		48	17.53 (13.05 – 29.98)	32.11 (26.47 – 37.30)	20	40
	EDTA	24	28.08 (13.57 – 41.20)	49.40 (38.91 – 57.85)	40	60
		48	11.98 (8.38 – 17.16)	27.23 (21.82 – 31.64)	20	40
	$Na_2S_2O_3$	24	14.17 (7.35 – 28.87)	34.34 (21.98 – 43.44)	40	60
		48	5.75 (32.25 – 10.32)	19.14 (15.35 – 23.25)	20	40
	C_{18}	24	34.74 (26.54 – 42.34)	49.38 (42.73 – 54.19)	40	60
		48	15.16 (10.88 – 22.49)	31.30 (25.34 – 36.27)	20	60
	CE	24	30.47 (21.12 – 38.66)	50.71 (43.84 – 56.59)	40	60
		48	28.72 (21.12 – 38.66)	50.71 (43.84 – 56.59)	40	60
Alexandra Basin	Initial	24	18.96 (15.22 – 25.81)	32.42 (28.64 – 36.52)	20	20
		48	17.06 (14.40 – 20.61)	24.59 (21.30 – 28.61)	40	40
	Baseline	24	24.90 (21.49 – 30.56)	37.43 (35.28 – 39.68)	20	40
		48	20.78 (16.67 – 32.03)	31.41 (28.58 – 36.30)	20	40
	EDTA	24	24.21 (22.15 -27.09)	37.73 (36.59 – 39.19)	20	40
		48	24.52 (22.54 – 29.42)	36.45 (35.31 – 38.61)	20	40
	$Na_2S_2O_3$	24	20.32 (18.19 – 22.97)	33.88 (31.82 – 35.39)	20	40
		48	18.22 (15.43 – 212.23)	31.91 (28.73 – 33.52)	20	40
	C_{18}	24	37.06)29.71 – 43.86)	58.09 (53.31 – 61.97)	40	60
		48	38.77 (31.06 – 47.02)	55.32 (50.60 – 59.47)	< 20	20
	CE	24	34.21 (32.25 – 35.52)	49.08 (47.33 – 19.76)	40	60
		48	33.12 (31.08 – 34.57)	48.11 (46.29 – 48.87)	< 20	20

[a] EC_{10} and EC_{50} values and corresponding 95 % confidence intervals in parentheses.
[b] NOEC, no observed effect concentration, the highest observed concentration at which no significant effect ($p \leq 0.05$) was detected
[c] LOEC, lowest observed effect concentration, the lowest tested concentration at which a significant ($p \leq 0.05$) effect was detected.