Ecotoxicology and Environmental Safety 73 (2010) 51–55

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Incorporation of 28-d *Leptocheirus plumulosus* toxicity data in a sediment weight-of-evidence framework

Blair G. McDonald [a,*], Cathy A. McPherson [a], Rachel DeWynter [a], Peter Kickham [a,1], Carrie Brown [b]

[a] Golder Associates Limited, 500-4260 Still Creek Drive, Burnaby, BC V5C 6C6, Canada
[b] Vancouver Fraser Port Authority, 100 The Pointe-999 Canada Place, Vancouver, BC V6C 3T4, Canada

ARTICLE INFO

Article history:
Received 1 April 2009
Received in revised form
13 August 2009
Accepted 17 August 2009
Available online 30 September 2009

Keywords:
Weight-of-evidence
Leptocheirus
Risk assessment
Statistical power
Sieving

ABSTRACT

A series of side-by-side trials were conducted to evaluate the variability of 28-d *Leptocheirus plumulosus* amphipod toxicity test data using existing and modified test protocols. One modification included examination of the influence of press-sieving on the sediment chemistry and the toxicity data. Press-sieving sediment did not reduce the variability in the toxicity data and also contributed uncertainty to the chemistry data. The second modification involved determining the sex of surviving adult amphipods so that the reproduction data could be measured as offspring/surviving female instead of only as offspring/surviving amphipod. Normalizing reproductive output to the number of adult females was ineffective in reducing the variability. The data from sediment toxicity tests are often interpreted in the context of 20% reductions and/or statistically significant reductions relative to negative controls. High inter-replicate variability makes default application of these decision criteria to the 28-d *L. plumulosus* toxicity test inappropriate regardless of whether or not samples are press-sieved or the sex of surviving amphipods is determined. This is not to say that the 28-d *L. plumulosus* toxicity test has no value for a sediment WOE: it provides long-term chronic data that may not otherwise be available. However, test-specific decision criteria must be established as part of the problem formulation based on the overall management goal, the availability of other lines of evidence (other toxicity tests as well as other types of data) and the desired level of certainty with respect to decision-making.

1. Introduction

A common approach in assessing the potential risks associated with contaminated sediments involves a weight-of-evidence (WOE) assessment (Chapman, 1990, 1996; Chapman et al., 1997; Chapman and Hollert, 2006) with a sediment toxicity testing component. The sediment toxicity component should cover a wide variety of organisms, life-cycles, exposure routes and feeding type (Chapman, 1990). The 28-d *Leptocheirus plumulosus* survival, growth and reproduction test (US EPA, 2001) is a candidate for marine and estuarine locations in addition to other tests routinely included in the WOE assessment (e.g., 10-d amphipod survival; 20-d polychaete survival and growth; 48 h larval development) McGee et al. (1993) commented the 28-d amphipod test is desirable because it provides evidence of subtle ecologically relevant effects being missed by traditional toxicity tests.

The decision criteria for interpreting toxicity test data in a WOE assessment focus on the magnitude of effects and/or statistically significant reductions in endpoint performance. Typical criteria used to evaluate toxicity data include one or more of the following: statistically significant reductions in mean response ($p>0.05$) relative to the negative control (Ingersoll and Macdonald, 2003); greater than 20% or 50% reductions relative to negative controls (Chapman and Anderson, 2005) or either of the above but with comparison to reference conditions. Decision criteria are often applied consistently irrespective of the specific toxicological endpoint under consideration.

However, in order to assign an appropriate "weight" to the data, the WOE should consider how the measurement endpoints (in this case, *L. plumulosus* survival, growth and reproduction) link to the underlying assessment endpoint (maintenance of a healthy benthic community). Previous sediment investigations using the 28-d *L. plumulosus* toxicity test (unpublished data) found high inter-replicate variability. We hypothesized that two test modifications would reduce the inter-replicate variability and thus improve the ability of the test to classify sediments within a WOE framework. According to the test protocol (US EPA, 2001), the negative control sediment is press-sieved (to remove indigenous amphipods and predators, but also creating a homogenous sample

* Corresponding author.
E-mail address: bgmcdonald@golder.com (B.G. McDonald).
[1] Presently employed by the British Columbia Ministry of Environment. The views contained in this paper are solely those of the authors and do not reflect official policies of the British Columbia Ministry of Environment.

0147-6513/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2009.08.007

without debris) while test sediments are not (US EPA, 2001). Notwithstanding the fact that press-sieving is not routinely recommended except to remove indigenous organisms or predators, the removal of debris from test samples (and creation of more homogenous samples) was hypothesized to result in lower inter-replicate variability. Also, reproduction endpoint is typically reported as offspring per surviving amphipod without consideration of the number of female amphipods present in the test container. Normalizing reproductive output to the number of surviving females was hypothesized to reduce the variability by factoring out the number of female amphipods in different replicates. Side-by-side testing of field-collected samples was conducted to evaluate these modifications.

2. Materials and methods

2.1. Sample collection and processing

Eleven surficial sediment samples were collected by divers from near-shore locations in an urbanized harbor (Burrard Inlet, Vancouver, BC) that was previously occupied by a ship repair facility and a sawmill. This location does not contain an indigenous *L. plumulosus* population. All samples contained shells, stones as well as woody and metallic debris associated with historical site use. Samples were well homogenized and split into two aliquots. One aliquot was press-sieved through a 500 μm sieve to remove the debris; the second aliquot was not sieved. Samples could not be processed through a 250 μm sieve because of the amount of debris present in the samples.

2.2. Chemical analyses

Chemical analyses were conducted by ALS Environmental (Vancouver, BC). For metals analysis, an aliquot of wet material was digested by microwave oven using a 1:1 ratio of nitric and hydrochloric acid and analyzed using an ICP-OES or ICP-MS (US EPA SW-846 Methods 6010B and 6020). Aliquots for PAH analysis involved an extraction with 1:1 mixture of hexane and acetone, which was then solvent exchanged to toluene and analyzed by capillary column GC/MS (Methods 3545 and 8270). Total organic carbon (TOC) was determined by high-temperature oxidation of carbon to carbon dioxide, which was measured with a non-dispersive infrared analyzer (Method 9060A). Unsieved samples were analyzed for other potential contaminants of concern (e.g., tributyltin, pesticides, PCBs and chlorinated hydrocarbons) as part of a broader sediment quality investigation but were not analyzed in the press-sieved samples because the primary focus of this study was on the response in the 28-d *L. plumulosus* toxicity test. Metals and PAHs were selected as representative contaminant groups for the purposes of evaluating the effects of press-sieving on the chemistry data.

2.3. Toxicity testing

Test organisms and negative control sediment (pre-screened through a 250 μm sieve) were provided by Chesapeake Cultures (Haynes, VA). Samples were tested in a side-by-side trial (sieved and non-sieved) in 1 L glass jars, each containing five replicates of 175 mL of sediment, approximately 725 mL of clean overlying seawater, and 20 neonate amphipods (US EPA, 2001). Samples were tested in two batches one day apart. The exposure period was 28 days at 25±2 °C under a 16:8 h light:dark photoperiod. Water quality measurements (pH, dissolved oxygen and temperature) were made, overlying water was renewed, and food was provided three times per week. The food ratio per replicate jar consisted of 1 mL of 20 mg/L Tetramin™ suspension during the first two weeks, and 1 mL of 40 mg/L Tetramin™ suspension during the first two weeks. Surviving adult amphipods and offspring were recovered on day 28 and preserved in ethanol. Surviving adults were examined using a dissecting microscope for distinguishable sexual characteristics (the presence of a notch on the first gnathopod appendage to identify males versus females; also, the presence of embryos within a brood pouch to confirm females). Sex was determined within 2 weeks of test termination and preserved adults were transferred to pre-weighed aluminum pans and dried at 60 °C for one day prior to measurement of weight to the nearest 0.01 mg using a calibrated Mettler AG104 analytical balance. Preserved offspring were also counted within 2 weeks. Subsamples of overlying and interstitial water for total ammonia (mg/L N) and interstitial water for total sulfide (mg/L S) were collected on days 0 and 28. A concurrent 96 h water-only reference toxicant test was conducted with cadmium. As part of the broader sediment investigation, samples were also assessed with other toxicity tests including a a 10-d amphipod (*Eohaustorius estuarius*) survival test following Environment Canada (1998) methods.

2.4. Data interpretation

Toxicity data were entered in ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA) to calculate mean and standard deviations. Standard deviations were evaluated using a paired two-sample *t*-test in Microsoft Excel to determine if there were statistically significant differences ($p>0.05$) in test endpoint variability between sieved and non-sieved samples, or between the different reproductive endpoints.

3. Results

Chemistry data for the sieved and non-sieved samples are summarized in Table 1. Total ammonia concentrations in interstitial water on days 0 and 28 ranged from 1.01 to 7.92 mg/L N while total ammonia concentrations in overlying water ranged from 0.011 to 3.37 mg/L N. Total ammonia concentrations were well below the recommended limit of 60 mg/L N for this species (Moore et al., 1997). Sulfide concentrations in interstitial water ranged from 0.03 to 70.0 mg/L S. Information regarding the tolerance of this species to sulfides was not available; however, one sample (A3) had a sulfide concentration in both sieved and non-sieved aliquots that was higher than the threshold proposed by Knezovich et al. (1996) for marine amphipods.

Survival, growth and reproduction (offspring/survivor and offspring/surviving female) results are summarized in Table 2. The sex ratio of the surviving adult amphipods was also calculated (Fig. 1). Both batches of samples met test acceptability criteria for negative control survival (≥80%), growth and reproduction (measurable) and water quality (salinity: 20±2 ppt; temperature: 25±2 °C) (US EPA, 2001). The 96 h cadmium reference toxicant test LC_{50}s were 0.60 mg/L Cd (95% confidence interval: 0.50–0.71 mg/L Cd) and 0.53 mg/L (95% confidence interval: 0.43–0.63 mg/L Cd), which fell within the control chart warning limits (mean±2 standard deviation; 0.42±0.29 mg/L Cd) from previous testing.

Paired two-sample *t*-tests were conducted on the standard deviations to determine if the method variations reduced the variability in the toxicological endpoints. The variability was not reduced in the sieved samples for any of the four toxicological endpoints. A statistically significant increase in variability was observed for sieved samples relative to the unsieved samples for survival (two-tailed $p=0.045$). There were no statistically significant decreases in variability in the sieved samples for the growth (one-tailed $p=0.33$), reproduction per surviving amphipod (one-tailed $p=0.24$) or reproduction per surviving female (one-tailed $p=0.12$) endpoints. A statistically significant increase

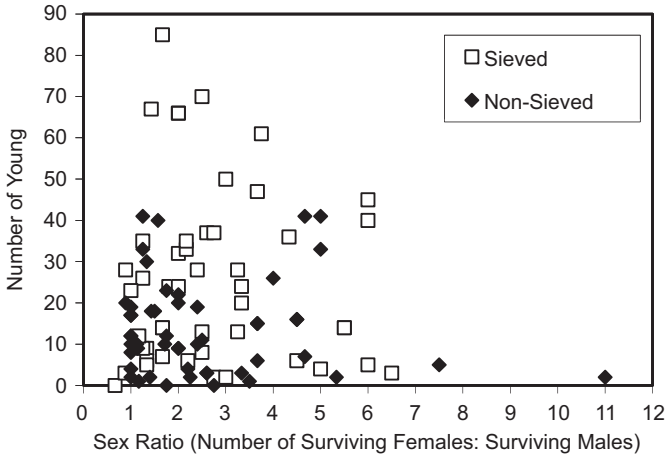

Fig. 1. Comparison of 28-d *Leptocheirus* reproductive output and sex ratio of surviving adults for individual replicates.