DEVELOPMENT AND APPLICATION OF CHRONIC AND SUBLETHAL SEDIMENT BIOASSAYS

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Introduction

Acute sediment bioassays can detect the toxic effects of severely polluted sediment. Many estuarine and coastal areas are moderately or lightly contaminated, and are unlikely to cause acute toxicity. They may still, however, exert toxicity in the longer term, therefore sensitive chronic sediment bioassays need to be developed to pick up sublethal effects of low levels of contaminants and/or persistent chemicals.

Chronic sediment bioassays should measure ecologically relevant endpoints which enable effects at the population level to be predicted. The selected test species should be sensitive, easy to culture, be tolerant to handling and a wide range of sediment types and have a short life cycle.

Chronic sediment bioassays have several potential applications in the UK:

- Regulatory Assessments - e.g. dredged material licensing
- Environmental Monitoring - e.g. UK National Monitoring Programme
- Toxicity Testing and setting Sediment Quality Criteria - e.g PARCOM

Chronic sediment bioassay protocols developed in the USA are not appropriate for use in the UK, for a number of reasons e.g. insensitivity, availability. As yet, the UK (or indeed Europe) has no definitive protocol for a chronic sediment bioassay.

Test Species and Test Methods

Three species were chosen for bioassay development:

1. *Corophium volutator*

- Estuarine amphipod
- Inhabits the top few cm of sediment
- Available all year
- Twice yearly reproduction

- 10-d acute sediment standard protocol (PARCOM)
- Chronic/sublethal assay = 28-d
- Endpoints = survival and growth
- Exposure System: 1L glass beakers, 2cm layer test sediment, overlying aerated seawater; 3 replicates per treatment; 10 juveniles per replicate
- Animals not fed, overlying water not renewed

2. *Leptocheirus plumulosus*

- Estuarine amphipod
- Inhabits the top few cm of sediment
- Not indigenous to the UK
- Easily cultured
- Short life-cycle (egg - egg < 25 days)

- Acute and chronic protocols developed in the USA
- Chronic test = 28 days
- Endpoints: survival, growth, reproduction
- Exposure System: 1L glass beakers, 2cm layer of test sediment, overlying aerated seawater; 3 replicates per treatment; 20 juveniles per replicate.
- Fed with a Tetramin slurry 3x weekly, overlying water renewed twice weekly

3. *Arenicola marina*

- Surface deposit feeding polychaete
- Available all year round
- Builds J-shaped burrows

- 10-d acute sediment standard protocol (PARCOM)
- Chronic/sublethal assay = 50+ days
- Endpoints: survival and casting
- Exposure system: 4 kg test sediment in plastic containers, layer of aerated overlying seawater; 2 replicates per treatment; 5 animals per replicate
- Animals not fed, overlying water not renewed

Research to Date

The acute sensitivity of *L. plumulosus* was firstly compared to *C. volutator* and *A.marina* in a series of 10 day bioassays, using a total of 41 environmental samples. Subsequently, acute and chronic/sublethal toxicity tests were carried out using **cadmium, tributyltin** (**TBT**), **phenanthrene** (a **PAH**) and **Aroclor 1260** (a **PCB congener**). Clean sediments were spiked with the test compound using standard techniques.

Results

Acute Toxicity Tests and Bioassays

Acute toxicity test results and a selection of the bioassay results are presented in Tables 1 and 2 respectively. Overall, the lugworm *A. marina* appears to be the most sensitive species in acute terms, and the amphipods had comparable responses. Survival of *L. plumulosus* in the field collected sediments was similar to that of *C. volutator*, the indigenous amphipod.

Table 1. 10 day LC50s obtained for the three test species and four test compounds.

Contaminant	*Corophium volutator*	*Leptocheirus plumulosus*	*Arenicola marina*
*Cadmium	24.18	14.57	2.54
*TBT	0.64	0.27	0.16
*Phenanthrene	51.38	40.86	nd
*Aroclor	nd	11.66	nd

* LC50s expressed as mg/kg dry sediment nd = not yet determined

Table 2. Percent mortality of the three test species in UK field sediments.

Site	*Arenicola marina*	*Corophium volutator*	*Leptocheirus plumulosus*
1	0	3	3
2	7	13	13
3	20	40	40
4	100	100	97

Discussion and Conclusions

Corophium volutator

- Increasing the exposure period for *C. volutator* from 10 to 28 days increased sensitivity of the survival endpoint by a factor of 3.
- Growth was not a more sensitive endpoint than survival in the long-term cadmium test; the LOEC and NOEC for both endpoints were the same.
- Results for the other test compounds with this species are currently being analysed.

Leptocheirus plumulosus

- In the cadmium test the NOEC and LOEC for growth and survival were the same. Both were significantly reduced at a concentration which was twice the value of the 10-d LC50.
- Discrepancies in survival may be due to the fact that the animals were fed during the chronic test and not in the acute test. Adding food may have reduced exposure to the contaminated sediment hence increasing the LC50.
- The phenanthrene results showed that growth was significantly reduced at the lowest concentration tested while survival remained high. In this instance growth was the most sensitive endpoint.
- Further tests with phenanthrene will be carried out to determine the NOEC for growth.

Arenicola marina

- The casting endpoint of this assay has potential for being sensitive enough to detect effects of low contaminant concentrations. Work is ongoing to assess the long-term effects of the above contaminants.

More research is required to investigate the complexities of nutrition during chronic sediment toxicity testing. Food may be a necessity for some test species in order for them to survive, grow and reproduce during the exposure period. Other species e.g. *C. volutator* seem to be able to grow adequately without food for 28 days.

Acknowledgment

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Chronic Toxicity Tests

Results of the chronic toxicity tests for cadmium using all three species are presented here, along with some preliminary results of the phenanthrene tests. Chronic tests with TBT and PCB are in progress. Statistical analyses were carried out using either one-way ANOVA or the non-parametric Kruskal-Wallis test.

C. volutator

- No effect on growth or survival (Figure 1) at cadmium sediment concentrations of 0.1 and 1 mg/kg.
- Survival was significantly reduced at a cadmium concentration of 5 mg/kg and above - the 28-d LC50 for survival was 8.5mg/kg ; growth was also reduced at these concentrations, but not significantly so.

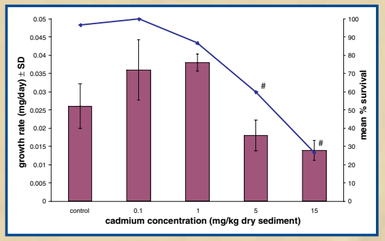

Figure 1. Survival and growth of C. volutator *exposed to cadmium-spiked sediment for 28 days. Significant differences from the control are denoted by * (growth) and # (survival) (p<0.05).*

L. plumulosus

- **Cadmium:** survival and growth were significantly reduced at 33mg/kg. No significant effect on survival, growth or reproductive output (Figures 2 and 3) below this. The 28-d LC50 for survival was 21.3mg/kg.
- **Phenanthrene:** significant reduction in survival and growth (Figure 4) at sediment concentrations of 35 mg/kg and above.
- At 20 mg/kg, survival was not significantly different from the controls, but growth and reproductive output (not shown) was significantly reduced i.e. there was a chronic sublethal effect at this concentration.

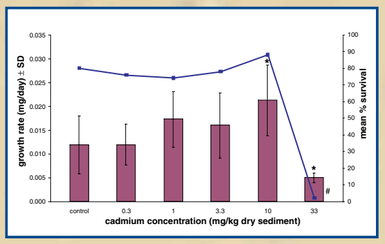

Figure 2. Survival and growth of L. plumulosus *exposed to cadmium-spiked sediment for 28 days. Significant differences from the control are denoted by * (growth) and # (survival) (p<0.05).*

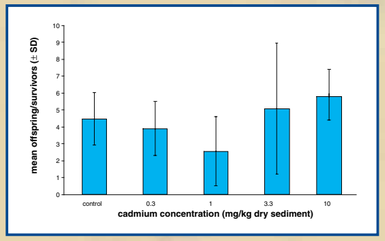

Figure 3. Reproductive output of L. plumulosus *exposed to cadmium-spiked sediment for 28 days.*

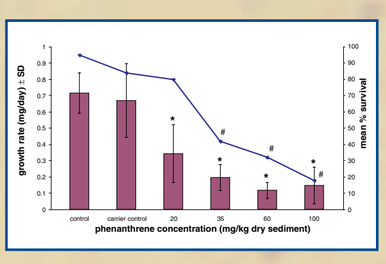

Figure 4. Survival and growth of L. plumulosus *exposed to phenanthrene-spiked sediment for 28 days. Significant differences from the control are denoted by * (growth) and # (survival) (p<0.05).*

A. marina

- Control and lowest two cadmium concentrations had good survival over 50-d (>80%)
- Poor replication in the higher concentrations led to significant effects on casting (Figure 5). A 50-d LC50 for survival could not be obtained.

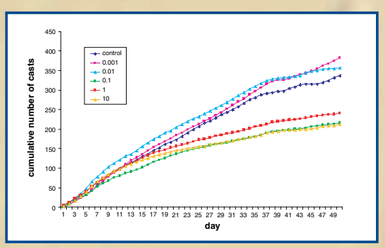

Figure 5. Effect of cadmium-spiked sediments on casting of A. marina *over a 50 day exposure period. Cadmium concentrations are expressed as mg/kg dry sediment.*

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