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Acute Toxicity of Cadmium to Two Species of Infaunal Marine Amphipods (Tube-Dwelling and Burrowing) from New Zealand

Christopher W. Hickey and David S. Roper

Water Quality Centre, DSIR Marine and Freshwater, P.O. Box 11 115, Hamilton, New Zealand

Chapman (1988) reviewed the range of species and different response levels used in sediment toxicity testing. He recommended that a battery of sediment bioassays incorporating different response criteria should be used in sediment toxicity evaluations as there is no single test which gives adequate assessment of sediment toxicity. However, one test which is becoming accepted as the 'benchmark' technique, and which is now commonly included in any battery of tests, is the amphipod bioassay. This test was developed in North America and uses the phoxocephalid *Rhepoxynius abronius* (Swartz et al. 1985). It has been subjected to rigorous interlaboratory evaluation (Mearns et al. 1986) and its sensitivity and usefulness has been demonstrated in a wide range of applications (Chapman 1988).

Unfortunately, species import controls make the use of existing test species (such as *R. abronius*) impractical for sediment toxicity testing in New Zealand. In view of the demonstrated sensitivity, reproducibility and value of the amphipod bioassay, we therefore initiated preliminary studies to assess the possibility of developing a test with a native New Zealand amphipod. Examination of intertidal sediments in several northern New Zealand estuaries has shown that the amphipods *Proharpinia hurleyi, Paracorophium excavatum* and *Paracalliope novizealandiae* can be found, at times, in large numbers (hundreds per square metre) (Authors' unpublished data). The results of benthic studies in Auckland's Manukau Harbour also suggested that *P. excavatum* and *P. hurleyi* may be sensitive to pollution, as these species were noticeably absent from sites where there was measurable sediment contamination (Roper et al. 1988; Fox et al. 1988). Using the protocol of Swartz et al. (1985), we undertook to test the sensitivity and laboratory performance of two local amphipod species; the tube-dweller *P. excavatum*, and the burrower *P. hurleyi*. It was anticipated that species with contrasting modes of existence may differ in their level of exposure to sediment contaminants and, therefore, show different toxic responses.

MATERIALS AND METHODS

Experiments were carried out using *P. excavatum* and *P. hurleyi* collected from the intertidal mudflats of Raglan Harbour (38° 49'S; 175° 57'E). The sediment used for these experiments was a fine-sandy mud, with 2.5% organic matter content

Send reprint requests to CW Hickey at the above address.

(loss on ignition at 400°C) collected from Raglan Harbour. "Enriched" organic content (7%) was achieved by addition of mud from the bottom of a dairy-shed oxidation pond. The animals were removed from the sediment by sieving through a 1 mm sieve. All animals were examined under a dissecting microscope prior to use, and injured and brooding female specimens excluded. Test animals were generally 1- 3 mm in length. All experiments were initiated within 48 hr of collection of the animals.

The experimental procedure followed the general method of Swartz et al. (1985), with slight modifications to incubation vessels and temperature. The incubations were performed in 100-mm diameter glass crystallizing dishes containing 10 mm sediment depth and overlain with 100 mL of 30‰ settled seawater with continuous aeration. Dissolved oxygen was maintained at > 7 mg/L. Ten amphipods were placed in each experimental container and there were four controls (since control survival was a major variable being investigated) and two replicates for each cadmium (Cd) concentration. Each container was examined daily and any dead specimens removed. All incubations were for 10 days at 20°C. The numbers of live amphipods were counted at the end of the experiment.

Salts of cadmium chloride were dissolved in distilled-deionized water to a concentration of 10,000 mg Cd/L. Test concentrations in sediments were achieved by addition of small (<1% of total volume) stock solution to a sediment slurry, followed by 5 min of vigorous mixing using a teflon spatula. The sediment surface was levelled, the supernatant water discarded, and clean overlying water added gently, so that there was no visible sediment disturbance. All of the treatments were then aerated overnight (15-20 h) at the incubation temperature prior to addition of the amphipods. Sediment concentrations were confirmed by replicate chemical analyses (APHA 1989 method: nitric acid digestion and atomic absorption) and the initial sediment concentrations used to calculate LC values. Replicate sediment concentration variability averaged 4.2% (range 1-7%) and measured Cd concentrations after 10 days averaged 6% less than the initial measurements (range 5-7%). Probit analysis was used to determine the 10-day LC50 (the concentration resulting in 50% mortality) values and 95% fiducial limits (Finney 1971).

Additional experiments were undertaken to determine the laboratory survival of *P. hurleyi* in response to physical factors including particle size, salinity, organic content, and "polluted" and "clean" sediment. Polluted sediment was collected from an area of Manukau Harbour which was known to be contaminated with various organochlorine compounds (Fox et al. 1988) and concentrations of heavy metals, suspected to have caused changes in invertebrate community structure (Roper et al. 1988). Clean sediment was obtained from Raglan Harbour, which has a relatively small catchment with minimal development. Unfortunately, insufficient numbers of *P. excavatum* were available to make this series of comparisons for that species.

RESULTS AND DISCUSSION

Field collection of potential test animals revealed that the occurrence of the three amphipod species (*P. hurleyi, P. excavatum* and *P. novizealandiae*) was inexplicably sporadic. This meant that returning to the same site after just a few weeks, the species which was dominant could then be rare, and another species would have replaced it as the dominant amphipod. *P. novizealandiae* were never present in sufficient numbers for use in these experiments. This inability to be able to find the same species consistently makes development of a routine technique very difficult, and highlighted the lack of ecological information on local amphipods. This uncertainty in the supply of amphipods could be overcome if laboratory cultures were established.

Table 1. The 10-day survival and LC50 results for amphipods in cadmium-dosed sediment. Cadmium concentrations on a dry weight basis.

Treatment	Species (mean survival %; SD)	
	Paracorophium excavatum (tube-dwelling)	*Proharpinia hurleyi* (burrowing)
Control (0.09 mg Cd/kg)	98 (5.0)	78 (15.0)
2.8 mg /kg*	100 (0.0)	-
6.0	100 (0.0)	70 (0.0)
8.4	95 (7.1)	60 (14.1)
12.1	85 (7.1)	80 (14.1)
23.3	-	60 (14.1)
70.0	0 (0.0)	-
LC50 (mean and 95% confidence interval)	18.3 (13.7-108.7) mg /kg	>23 mg /kg
LC10	10.6 (7.2-14.8)	-

- indicates not measured
*measured Cd concentrations at initiation of experiment

Table 2. The 10-day survival results for *Proharpinia hurleyi* in response to physical factors and a test sediment

Treatment	Species (mean survival %; SD)
	Proharpinia hurleyi
Control (Raglan mud)	78 (15.0)
Sand:	
very fine sand	10 (-)
fine sand	40 (-)
medium sand	10 (-)
coarse sand	10 (-)
Salinity (Raglan mud):	
16‰	70 (-)
8‰	40 (-)
"Enriched" sediment (organic content 7%)	100 (-)
"Polluted": "Clean" sediment:	
15:85	75 (35.4)
50:50	85 (21.2)
100:0	35 (49.5)

- indicates not measured in replicate

The control and cadmium survival values together with calculated 10-day LC50 values are summarized in Table 1. The control survival of *P. excavatum* was good, exceeding 90% in all replicates; however, this contrasted with the lower, and more variable survival of *P. hurleyi,* which averaged 78%. Sediments dosed with cadmium showed that *P. excavatum* was possibly slightly more sensitive (LC50 = 18.3 (13.7-108.7) mg Cd/kg (DW)) than *P. hurleyi,* though the poor control survival and only limited mortality at high cadmium concentrations precluded calculation of the LC50 concentration (LC50 > 23 mg Cd/kg (DW)) for *P. hurleyi.*

These LC50 values are somewhat higher than the average value of 9.8 mg Cd/kg (DW) (CV 12.04%) found by Mearns et al. (1986) for an interlaboratory study using *Rhepoxynius abronius* and cadmium-dosed sandy sediments (98% sand; 1.0% total volatile solids). Di Toro et al. (1990) found an LC50 value of 290.0 mg Cd/kg (DW) for *Rhepoxynius hudsoni*, while *Ampelisca abdita* LC50 values ranged from 1,070 to 2,850 mg/kg (DW). Published comparative data for different amphipod species is not available for standard contaminants in the same sediment type, but is limited to solution exposures.

Hong and Reish (1987) compared the relative sensitivities of 5 amphipod species to cadmium solutions and found a 5-fold range in sensitivities. This suggests that the results observed here, in comparison with the sediment results for *R. abronius*, are within the range which might be expected for amphipod species. Additionally, Hong and Reish (1987) found that *R. abronius* was the most sensitive of the species tested (LC50 = 0.24 (0.19-0.32 95% CI) mg Cd/L for a 96-h exposure). Even for this species large variations have been found in its sensitivity. For example, this LC50 value of 0.24 mg/L for *R. abronius* is 5-fold less than the LC50 value of 1.61 mg/L found by Swartz et al. (1985) for the same species. Hong and Reish (1987) suggested that these differences may be attributed to differences in collection and transportation of test animals, and different laboratory conditions. Rather than species differences, it is possible that field and laboratory procedures may have a greater influence on test results. This emphasizes the need for rigorous standardization in the protocol.

Differences between test results have been linked to sediment variables such as particle size (DeWitt et al. 1988), organic carbon (Swartz et al. 1988) and acid volatile sulphide (AVS) (Di Toro et al. 1990). This suggests that it is particularly important to understand the response of test species to natural environmental variables, and the way in which these variables can modify toxicity. Di Toro et al. (1990) showed that AVS normalization could account for over an order of magnitude difference in cadmium LC50s. The sediments used in this study were collected from surficial layers which appeared to be oxidized and, hence, would be expected to contain little or no AVS.

Kemp and Swartz (1988) found that the acute toxicity of cadmium to *R. abronius* appeared to be due principally to cadmium dissolved in the interstitial water rather than whole sediment concentrations. Di Toro et al. (1990) also support a correlation between mortality and interstitial water concentrations. In addition to any inherent physiological differences in the toxicological sensitivity of our two test species, it was expected that there may have been significant differences in their test responses as a result of their different modes of existence. During the experiments *P. hurleyi* was observed to continually burrow, with 10 organisms completely turning-over the sediment surface of a 100-mm diameter dish overnight. *P. excavatum*, by contrast, established tubes which they maintained over the 10-day experiment, and caused only minimal surface disturbance.

Conceivably, *P. hurleyi* in burrowing through the sediments would suffer from greater exposure to sediment-bound and interstitial contaminants, compared with *P. excavatum* which occupies a permanent tube which it irrigates with the overlying water. This trial failed to show any major difference in the sensitivity to Cd between the species.

The preliminary results given in Table 2 show that mortality of *P. hurleyi* was increased with both fine and coarse sand but that there was good survival in organically enriched mud as expected. Exposure to mixtures of "polluted" and "clean" mud gave variable results, but indicated that *P. hurleyi* is sensitive to the contamination present in Manukau Harbour mud. Results of the salinity exposure trial were inconclusive, but it appears *P. hurleyi* is a euryhaline species, which is to be expected for an estuarine amphipod. Overall, these results suggest that this species is very sensitive to changes in sediment particle size, which severely limits its usefulness for routine testing. Also, the fact that survival was 100% in organically enriched mud, compared with mean survival in control mud of only 78%, might indicate that *P. hurleyi* may be sensitive to food limitation. Again, this factor would limit its usefulness as a test animal, unless feeding for the duration of the test was carried out.

These preliminary studies indicate that development of an infaunal amphipod test using a New Zealand species may be possible, but that there are several problems to overcome. Future work could focus on the possibility of culturing amphipods in the laboratory, to overcome problems caused by their sporadic abundance in the field, and further testing of amphipod sensitivity to a range of known pollutants and natural sediment characteristics. Whether tube-dwelling or burrowing species are more appropriate for determining toxicity under various sediment conditions remains to be clarified.

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