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New Method for the Assessment of Contaminant Uptake Routes in the Oligochaete *Lumbriculus variegatus*

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A variety of toxic chemicals are known to accumulate in sediments because of their hydrophobic character. Toxic effects on benthic organisms result from a combination of direct exposure to sediment particles, uptake from sediment pore water and overlying water as well as from ingestion of sediment particles. Many current approaches for modelling sediment exposure assume that benthic organisms are primarily exposed to a contaminant in the sediment pore water (Di Toro *et al.,* 1991; Thomann *et al.,* 1992 and Belfroid *et al.,* 1995). However, previous research has shown that some contaminants are accumulated from direct contact of the organism with sediment particles and via ingestion (Boese *et al.,* 1990 and 1996; Landrum, 1989, Loonen *et al.,* 1997 and Kaag *et al.,* 1997).

In order to understand the relative importance of different pathways of uptake one must examine each route of uptake individually. More in-depth knowledge of relative routes of uptake will help in developing more precise sediment accumulation models and aid in the risk assessment process. It is, however, difficult to satisfactorily study the relative contribution from different uptake routes.

Leppaenen and Kukkonen (1998) have investigated the significance of ingestion as an uptake route using the oligocheate worm *Lumbriculus variegatus.* This species reproduces by architomy, in which new individuals bud off the anterior end of the parent and after fragmentation, individuals regenerate fresh segments for tail (anterior end) and head (posterior end). The formation of a new head takes 6-7 days during which time the worm is unable to feed. This characteristic enabled the authors to expose feeding and non-feeding worms to sediment contaminated with the PAH pyrene and monitor body burden.

Isolating and identifying recently budded non-feeding worms is, however, a skilled and very labour-intensive exercise requiring the separation of a large number of worms from sediment. It is therefore impractical to carry out intensive uptake tests

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requiring the use of large numbers of worms (up to 300 worms are typically needed for each uptake test). It is also difficult to determine the precise age of headless individuals which are used in the sediment test. Ideally non-feeding worms should be freshly fragmented to prolong the non-feeding phase.

This short communication describes a new method for performing feeding and non-feeding tests by removing the heads from *L. variegatus.* Decapitation provides a viable organism which is unable to feed for the 7 day period during which a new head generates. This method is currently being employed to performuptake tests for a number of organic contaminants. Preliminary data are presented here to demonstrate the validity of the new technique.

MATERIALS AND METHODS

L.variegatus was cultured in house and originated from a brood stock supplied by the USEPA, Midcontinent Ecology Division, Duluth, MN. The culture was reared in a 57 L glass aquarium in a constant flow (approx. 25 mL/min) of ground water in a 16:8 1ight:dark cycle. Shredded and pre-soaked paper towels were used as substrate. The cultures were fed three times a week with 4 g of finely ground tetramin (Tetrawerk, Germany) fish food.

Test worms were retrieved with a glass pipette from a vessel containing around 500 worms which had been randomly selected from the culture. Intact adult worms (ie, fully formed posterior and anterior ends) weighing between 5-10 mg were used in the experiment. Headless worms were prepared by removing the head end of the worm (2-4 mm) with a scalpel. The head end of the worm can be identified as it is slightly thicker and greener than the tail end.

In the first experiment, headed and headless worms were exposed to diclorophenol (DCP) in a water only and a water-sand system. The goal was to establish if the magnitude of uptake differed in headed and headless individuals. Twenty-four glass jars were filled with 30 mL of spiked ground water (100 Bq/mL of ring-labelled ^{14}C- DCP, specific activity 1.5 MBq/mg). Clean sand (10 g, <100 μm) was added to 12 of the test systems and ten worms were placed in each jar. Six replicates of headed and headless worms were prepared for the water and water-sand system.

After a 5 day test period the worms were removed from the spiked water, blotted dry with tissue paper, weighed and placed in a liquid scintillation counter (LSC) vial with 2 mL of tissue solublizer (Soluene, Canberra Packard) and allowed to

dissolve overnight. Next day, 10 mL of LSC cocktail (Hionic Flour, Canberra Packard Ltd) was added to the vial and mixed. Test solution (1 mL) was placed in 10 mL of LSC cocktail (Instagel, Canberra Packard Ltd). Samples were counted in a Beckmann LS6000SE LSC. The data was corrected for quenching using parallel standards prepared in the same matrix after correcting for background.

In the second experiment, a sediment exposure test was carried out to assess the significance of ingestion as an uptake route for pyrene. Clean sediment (collected from ARC Study Centre, Milton Keynes, 1.7% organic carbon, 73% <63 μm, 50% water content) was spiked with pyrene (^{14}C ring-labelled, specific activity 10.7 MBq/mg) to give a sediment concentration of 800 Bq/g dry weight. To spike the sediment, the test compound was added dropwise in a methanol solution to the sediment. The sediment was then thoroughly mixed for an hour and left overnight to equilibrate.

Thirty glass jars (60 mL) were prepared by adding either 6 headed or six headless worms to each jar. Spiked sediment (20 g) was added to each vessel covering the worms. Each vessel was topped up with 30 mL of WRc groundwater and the jar sealed and fitted to an aeration system venting into a solution of 2 M sodium hydroxide. Three replicates of each headed and headless worms were removed after 2, 4, 6, 8, and 12 days.

At each time interval the worms were removed from the sediment and depurated overnight in a beaker of groundwater and sand (10 g) to remove any sediment particles contained in the gut of the feeding worms. After depuration the worms were analysed as above. At each time interval the overlying water concentration and sediment concentration was analysed. Sediment was extracted in 4 mL of methanol by ultrasonification for 60 min, followed by centrifugation. The supernatant was added to 10 mL of Instagel and counted on the LSC.

The data from the first experiment were analysed by comparing the means of the headed and headless worm concentration for the water only and the water and sand exposure using a t-test. A similar approach was used in the second experiment, body burdens in feeding and non-feeding worms were compared for each time period using a t-test.

RESULTS AND DISCUSSION

Figure 1 presents the bioaccumulation factors (concentration in worms [Bq/g wet weight] divided by concentration in water [Bq/mL]) for the water and water-sand

test performed with ^{14}C-DCP. The data shows that there is no significant difference (t-test, T=-0.7, df=5, p=0.05 (water-only), T=0.8, df=5 p=0.05 (sand and water)) in the bioaccumulation factor (BAP) between headed and headless worms in either the water-only or water and sand exposure. The slightly higher BAF measured for the samples containing sand can be explained by small amount of adsorption of the ^{14}C-DCP to the particulate phase. This data confirms that headed and headless worms behave similarly in water-only tests where the sole route of exposure is by uptake from the aqueous phase.

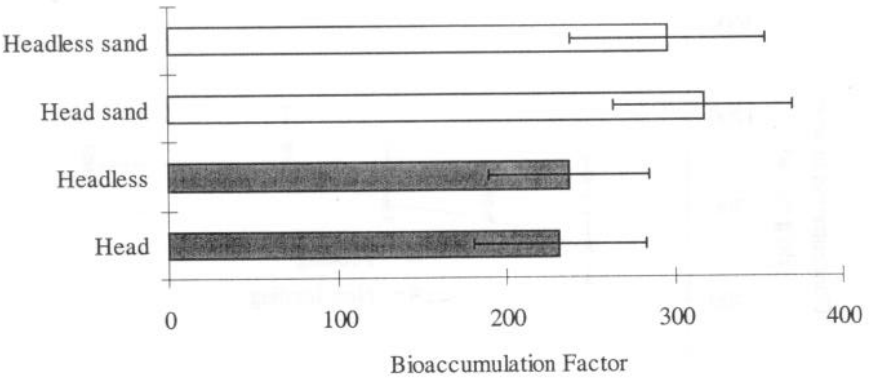

Figure 1. Comparison between bioaccumulation factors for ^{14}C-DCP in headed and headless *Lumbriculus variegatus* in water only tests. Error bars are 95% confidence intervals.

Figure 2 shows the variation in uptake observed for ^{14}C-labelled pyrene between feeding and non-feeding worms. At day 2 (t-test, T=4.5, df=2, p=0.05) the feeding worms exhibit distinct enhancement in uptake with respect to their non-feeding counterparts. By day 8 (t-test, T=0.6, df=2, p=0.05), the level of accumulated pyrene in both groups was similar. After 7 days, the headless worms regenerate a new head and start ingesting sediment which accounts for the observed increase in uptake. Concentrations of pyrene in the sediment and overlying water remained constant throughout the experimental period.

The main routes of uptake for pyrene are by sediment pore water and direct contact with sediment particles, however ingestion of sediment accounts for approx. 20% of the body burden in the feeding worms. Leppaenen and Kukkonen (1997) also found that non-ingesting worms accumulated less pyrene. Similarly, with the onset of feeding in this group an increase in body burden was observed. The ability to carry out feeding and non-feeding tests on *L. variegatus* by simply removing the

heads at the start of a bioaccumulation test, avoids the labour-intensive selection of worms from a culture at the appropriate life-cycle stage. The technique makes the use of *L. variegatus* as a test organism for investigating the different routes of uptake of organic contaminants a much more practical proposition.

This methodology is currently being used to assess the differences in routes of uptake for 27 organic compounds (including pesticides, chlorophenols and PAHs) into *L. variegatus.*

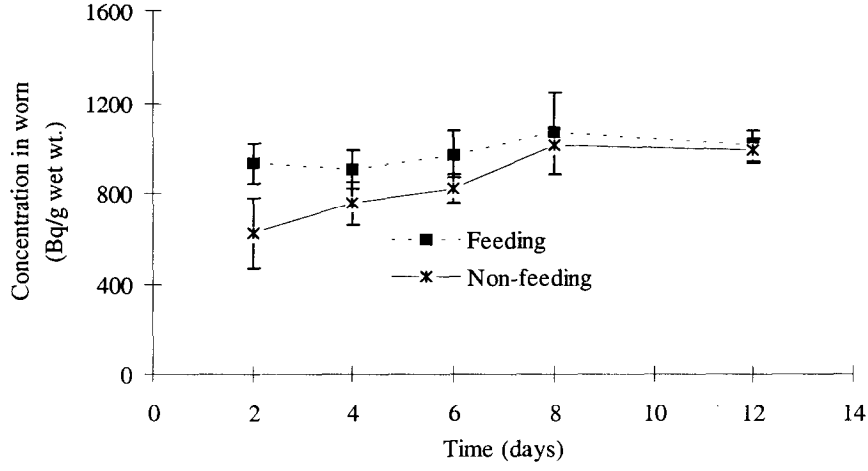

Figure 2. Variation in uptake of ^{14}C-pyrene in feeding and non-feeding worms during 12 days of incubation. Error bars are 95% confidence intervals.

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