Bull. Environ. Contam. Toxicol. (1989) 43:350–354

Preliminary Investigation of Protein Utilization by an Aquatic Earthworm in Response to Sublethal Stress

Timothy J. Keilty and Guy R. Stehly

Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105-1593, USA

Previous studies have illustrated the potential usefulness of monitoring biomolecule levels (total protein, DNA, and RNA) in juvenile fish and invertebrates exposed to sublethal doses of environmental toxicants. Ninety-six hr exposures of five contaminants (benzophenone, ethyl acetate, hexavalent chromium, hydrogen cyanide, and p-cresol) to larval fathead minnows yielded measurable alterations in total protein, DNA, and RNA that closely paralleled results from concomitant 28–32 d growth assays (Barron and Adelman 1984). *Daphnia magna* exposed to toxicants in various assays also exhibited changes in protein, RNA, and DNA levels, with the most sensitive responses usually occurring during the rapid growth life stage (McKee and Knowles 1986a, b; Knowles and McKee 1987). These results suggest that the use of biomolecule analyses as indicators of reduced growth in chemically stressed aquatic organisms holds promise.

Over the past several years, we have conducted both acute and sub-acute sediment bioassays using endrin-contaminated Great Lakes sediments and the aquatic earthworm *Stylodrilus heringianus* (Keilty *et al.* 1988a, b, c). Because growth, or the lack of it (measured as the change in dry worm body weight over an experiment), has proved to be a reliable indicator of sublethal stress, the relationship of total protein to dry body weight was investigated in this preliminary study. Total protein was chosen because it is relatively easy to measure (a spectrophotometer and commercially available protein reagent packages are all that is needed), is a major component of dry weight and is less susceptible to breakdown or interferances during analysis (Barron and Adelman 1984).

MATERIALS AND METHODS

Sediments and worms were collected in Lake Michigan approximately 10 km offshore from St. Joseph, Michigan, in 42 m of water. Sediments were dried at 60 °C, passed through a 0.25 mm sieve, and reconstituted with lake water. Worms were stored at 10 °C in lake sediments for 1 mon prior to use.

send reprint requests to Timothy J. Keilty at Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Blvd., Ann Arbor, Michigan 48105.

Guy R. Stehly present address – Armstrong Aerospace Medical Research Laboratory, Building 79, Area B, Wright-Patterson Air Force Base, Ohio 45433

The chlorinated pesticide endrin was selected because of previously determined toxicity test data for endrin–spiked Lake Michigan sediment. The experimental concentration of 50 μg/g dry weight sediment (nominal) was chosen to represent the high range of concentrations where sublethal behavioral or feeding responses were previously observed (see Keilty *et al.* 1988a).

Endrin was added to 120 g dry weight sediment (acetone carrier, 1 mL) in 2 L lake water. The mixture was mechanically stirred for 24 hr and allowed to settle for 72 hr. Overlying water was aspirated off, and enough fresh lake water added and remixed with the sediment to fill six 50–mL beakers (approximately 20 g sediment, 30 mL water/beaker). Beaker contents were allowed to settle for 72 hr at 10 °C. Groups of ten adult (approx. 25 mm) *Stylodrilus heringianus* were then added randomly to each beaker. Control sediments were dosed with 1 mL acetone carrier and treated in the same manner. Test beakers were stored in the dark in an environmental chamber maintained at 10 °C for 69 d or until sampled. Aerated lake water was gently pipetted into beakers to compensate for evaporation over the experiment.

Worms were examined from a control and an experimental beaker at day 4, 8, 20, 28, 39, and 69. Worms were removed and cleaned of debris, then dried in a dessicator to constant weight, in lots of twos and threes from each beaker (n=4/beaker). Worms were homogenized with water in a small all–glass tissue grinder, and an aliquot of homogenized worm tissue was used for protein analysis. The worm homogenate and BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, Illinois) were incubated for 30 min at 60 °C, and protein levels were determined spectrophotometrically (562 nm) using a Bausch and Lomb Spec 710 (Rochester, New York). Bovine serum albumin was used as a standard for the protein standard curve.

Effect of sediment–borne endrin on percent protein (μg protein/mg dry weight of worm), amount of protein/worm, and worm dry weight were analyzed by two–way analysis of variance (α=0.05). Significant differences were determined by Duncans multiple range test.

RESULTS AND DISCUSSION

Protein levels (% protein) in oligochaetes from endrin–spiked sediments did not significantly change over the 69–d test period (Table 1). Body dry weights of worms in the contaminated sediment did, however, decrease with prolonged exposure (Table 1). Because of this reduction in body weight, the relative percent of protein significantly increased with exposure (Fig. 1). This suggests that other potential energy sources (most likely lipid material) were being utilized. Other research has determined that the total lipid content of *Stylodrilus heringianus* ranges from 12–19% seasonally (Gardner *et al.* 1985). Such lipid stores apparently are capable of supporting the organism over relatively long periods of chemical stress where feeding activity has been reduced. We observed that the worms remained mostly on the surface of endrin–spiked sediments, while worms in control sediments remained burrowed for the entire experiment. This burrowing avoidance response was similar to previously observed behavior (Keilty *et al.* 1988a). Although we did not continue the experiment past 69 d, it is possible that protein would eventually be metabolized. In this event, the worms would probably be close to death.

Body weights in control worms increased significantly over the experiment (Table 1). Because the relative percent of protein did not increase, (Fig. 1) it is suggested that the organisms were storing fats and were not stressed during the experiment.

Table 1. Temporal mean (s.d., n=4) dry weight (mg) and protein per worm (μg/worm) of *Stylodrilus heringianus* in control and endrin–spiked sediments.

	Dry weight (mg)		μg protein/worm	
Time	Control	Endrin	Control	Endrin
4 d	0.551 (.080)	0.515 (.142)	234 (26.1)	222 (47.5)
8 d	0.581 (.078)	0.448 (.092)	237 (18.8)	245 (41.1)
20 d	0.633 (.212)	0.442 (055)	257 (57.4)	255 (27.8)
28 d	0.766 (.122)[1]	0.405 (.110)[2]	309 (28.9)[1]	230 (69.5)[2]
39 d	0.824 (.171)[1]	0.389 (.074)[2]	291 (50.6)	249 (50.9)
69 d	0.740 (.152)[1]	0.281 (.076)[2]	323 (57.2)[1]	201 (44.0)[2]

[1] significantly different from 4 d control value
[2] significantly different from control value at same time

In previous tests where decrease in biomolecule levels (compared to controls) predicted chemical stress, the greatest sensitivity was observed in individuals in a rapid growth phase (Knowles and McKee 1987; Barron and Adelman 1984). In the present experiment protein growth was not rapid. Significant differences in the amount of protein between control and endrin exposed worms were detected only at 28 and 69 d (Table 1). For practical reasons, only adult oligochaetes are used in our bioassay procedures, and it is possible that significant decreases in protein at earlier times would be obtained if juvenile, rapidly growing oligochaetes, were employed.

The combination of growth in the control worms and loss of body weight in the endrin exposed worms with time resulted in a divergence of percent protein (Fig. 1). Percent protein in endrin–spiked sediment worms was significantly higher than percent protein in control worms for all data points after eight days, whereas body weight and absolute amount protein were not. This suggests that percentage of dry body weight as protein may be a more sensitive measure of sublethal exposure to sediment toxicants in adult worms.

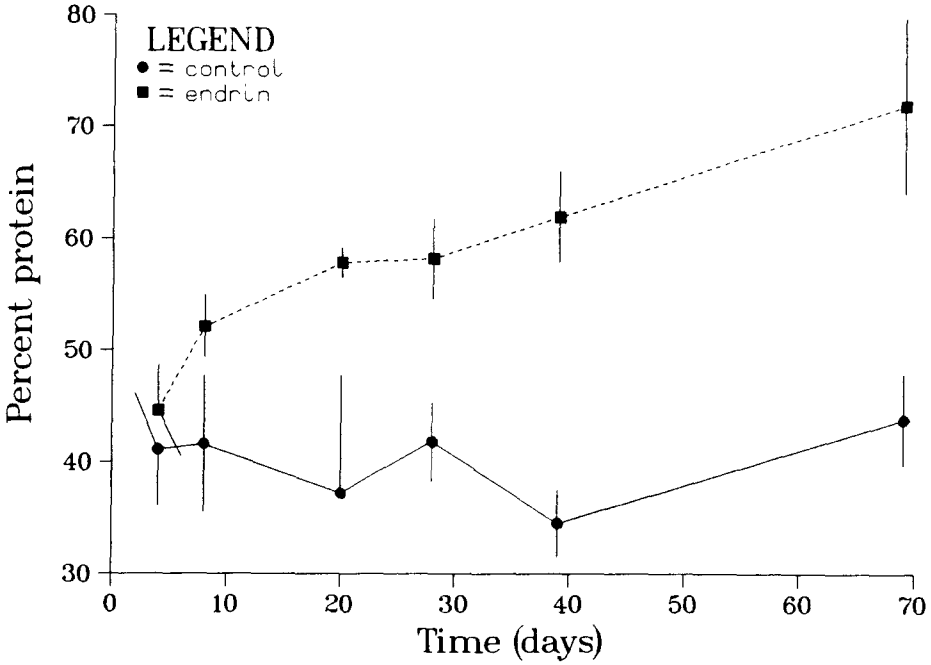

Figure 1. Relative percent protein of total body weight of *Stylodrilus heringianus* in control and endrin-spiked sediments during experiment; significant difference $p<0.05$, at all times after 4 days. Error bars are standard deviations of four observations.

In conclusion, sublethal responses to endrin-contaminated sediments can be monitored by changes in percentage of protein in control vs endrin exposed worms or by worm body weight. The measure of total protein does not appear to be as useful in this regard. It does, however, provide insight on the biochemical mechanism(s) of metabolism under stressful conditions.

Acknowledgments. We thank P. F. Landrum and M. A. Quigley for critical review of an earlier version of this manuscript. This is GLERL contribution number 649.

REFERENCES

Barron MG, Adelman IR (1984) Nucleic acid, protein content, and growth of larval fish sublethally exposed to various toxicants. Can J Fish Aquat Sci 41:141–150

Gardner WS, Nalepa TF, Frez WA, Cichocki EA, Landrum PF (1985) Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. Can J Fish Aquat Sci 42:1827–1832

Keilty TJ, White DS, Landrum PF (1988a) Short-term lethality and sediment avoidance assays with endrin-contaminated sediment and two oligochaetes from Lake Michigan. Arch Environ Contam Toxicol 17:95–10

Keilty TJ, White DS, Landrum PF (1988b) Sublethal responses to endrin in sediment by *Limnodrilus hoffmeisteri* (Tubificidae), and in mixed-culture with *Stylodrilus heringianus* (Lumbriculidae). Aquat Toxicol 13:227–250

Keilty TJ, White DS, Landrum PF (1988c) Sublethal responses to endrin in sediment by *Stylodrilus heringianus* (Lumbriculidae) as measured by a ^{137}Cesium marker layer technique. Aquat Toxicol 13:251–270

Knowles CO, McKee MJ (1987) Protein and nucleic acid in *Daphnia magna* during chronic exposure to cadmium. Ecotox Environ Safety 13:290–300

McKee MJ, Knowles CO (1986a) Protein, nucleic acid and adenalyate levels in *Daphnia magna* during chronic exposure to chlordecone. Environ Pollut Ser A42:335–352

McKee MJ, Knowles CO (1986b) Effects of fenvalerate on biochemical parameters, survival, and reproduction of *Daphnia magna*. Ecotox Environ Saftey 12:70–84.

received January 5, 1989; accepted March 6, 1989.