Bull. Environ. Contam. Toxicol. (1997) 58:387-393


# Rapid Toxicity Estimation Using Soil Ciliates: Sensitivity and Bioavailability

J. R. Pratt, D. Mochan, Z. Xu

Environmental Science and Resources, Portland State University, P.O. Box 751, Portland, Oregon 97207-0751, USA

Received: 28 May 1996/Accepted: 3 November 1996

Several characteristics of soil ciliates make them ideal toxicity test organisms. Ciliates are ubiquitous in ecosystems and play major roles in decomposition by cropping bacteria. Many ciliates form resistant resting stages, usually cysts that allow the organisms to remain dormant during adverse conditions (e.g., drying) and then to exploit acceptable conditions rapidly. Growth rates can be high (3-4 doublings per day). Although many of the most opportunistic species are poor competitors (Foissner et al. 1991), the ability of these organisms to grow quickly from resting stages can be exploited for toxicological purposes. Opportunistic or pioneer species have been shown to be more sensitive to toxic chemicals than the slower growing species that comprise the remainder of communities (Ruthven and Cairns 1972, Hart and Cairns 1984).

Previous studies have shown the utility of ciliates in assessing toxicity. Most of these studies have concentrated on the response of the ciliate *Tetrahymena pyriformis* to a variety of toxicants (e.g., Roberts and Berk 1990), and the *T pyriformis* model has been used in developing quanitative structure-activity relationships (e.g., Schultz et al. 1994). Ciliate behavior has been used to assess the effects of metals (Berk et al. 1985). Recently, a growth test using the ciliate *Colpidum campylum* has been developed and tested in several laboratories (Dive et al. 1990). An essential feature of effective assessment tools is the ability to simulate field exposure (Forbes and Forbes 1994). For many evaluations, laboratory testing requires shipping and holding field samples, reducing environmental realism. Test procedures that minimize culturing enable testing at or near field sites with minimal laboratory facilities.

We examined the sensitity of a *C. inflata* growth test to different toxic metals and evaluated the effects of test media with differing dissolved organic carbon content to assess bioavailability of the tested toxicants.

*Correspondence to:* J. R. Pratt

## METHODS AND MATERIALS

The test species was *Colpoda inflata*, a common ciliate (40-60 um long). Cells were isolated from oak litter (Centre County, Pennsylvania, USA), and dry cysts (ATCC 30917) were obtained from the American Type Culture Collection (Rockville, MD, USA). Cultures were developed from stored cysts and were maintained for 2-7 d in 10% Sonneborn's *Paramecium* medium. Sonneborn's medium was prepared by boiling 2.5 g cerophyll (cereal leaves) for 5 min in 1 L of distilled, deionized water. The mixture was filtered (#1 Whatman filter paper), the volume was adjusted to 1 L with distilled water, and 0.5 g $Na_2HPO_4$ was added. The full-strength medium was diluted prior to use with distilled, deionized water, and was autoclaved in 50 ml aliquots. The food bacterium was a non-pathogenic strain of *Klebsiella pneumoniae* (ATCC 27889).

Toxicity tests were conducted in sterile, 24-well, polystyrene tissue culture plates (Costar, Inc.) using either 10% Sonneborn's medium or a minimal salts medium consisting of 6 mg KC1, 4 mg $CaHPO_4$, and 2 mg $MgSO_4$. Sterile medium was dispensed into wells and amended with toxicant from stock solutions of reagent grade chemicals prepared in sterile distilled, deionized water. Dilution of the test medium by added toxicant was never more than 10%. After medium and toxicant were dispensed into test wells, ciliates were added from log-phase cultures (48-96 hr old) along with food bacteria. The volume of culture added assured that equal numbers of ciliates (approximately 100) were added to each well.

Experiments were conducted to estimate the sensitivity of ciliate growth to the toxic metals cadmium, copper, and zinc. Each experiment consisted of four replicates of five test concentrations plus controls. The toxicity of cadmium, copper, and zinc was evaluated using diluent media of Sonneborn's medium (5%, 10%) or the minimal salts medium. The 10% Sonneborn's medium had 40 mg/L of dissolved organic carbon.

The test period was 24 hr. After 24 hr, subsamples were removed from each test well and enumerated using a direct counting technique as follows. Each well was thoroughly mixed with a micropipettor, and a 20 ul subsample was removed and transferred to a clean microscope slide as 3 or 4 drops. All of these drops were then immediately scanned at low magnification (40 x) on a stereomicroscope to search for active cells. Active cells are always moving and can be easily distinguished from bacterial aggregates or cysts. For a given well, the subsampling techniques were repeated at least three times to assure that an accurate estimate of the population in the well had been obtained. If repeat counts of subsamples varied by more than 30%, subsampling continued until population estimates stabilized. This procedure was repeated for each well, and the mean of subsample estimates for a given replicate was used in later analyses.

Data were examined by estimating the toxicant concentration corresponding to a

50% inhibition of growth (IG50) relative to controls. These analyses were done by regressing cell number on log dose and then using inverse prediction (Sokal and Rohlf 1981) to estimate the IG50 from the control response (mean= 100%).

## RESULTS AND DISCUSSION

Ciliate growth was sufficiently rapid to produce large numbers of cells in a short time period. Generally, controls grew from the initial innoculum of about 100 cells/ml to over 500 cells/ml in 24 hr. experiments. Three investigators produced median values for control growth of 450, 470, and 550 cells/ml for 15 growth experiments. Variability of the control response within an experiment ranged from 8.6 to 30.8% as coefficient of variation.

Experiments using different species and strains of *Colpoda* showed that sensitivity varied widely. Initial tests of copper toxicity in 10% Sonneborn's medium (highly dissolved organic matter) showed greatest sensitivity of the ATCC strain and revealed that this strain was much more sensitive than earlier tests of copper toxicity to *C. steinii* in inorganic media (Forge et al. 1993).

Results of the several independent toxicity tests showed that the order of estimated toxicities was Cd>Cu>Zn (Table 1). This order corresponds to the ranking of these three metals by national water quality criteria in the United States (USEPA 1986) based on acute and chronic toxicity studies of standard fish and invertebrate test species. The IG50 estimates were in the lower end of reported acute toxicity estimates and in the higher range of chronic toxicity estimates, suggesting that these growth tests have sensitivity intermediate between standard acute and chronic tests.

Organic media were expected to affect the bioavailability of metals, and this pattern was evident for tests of cadmium and copper toxicity (Table 1, Fig. 2). For zinc, increasing the concentration of dissolved organic carbon in the medium had less effect. Organic media are necessary for growth of cultures, but *C. inflata* can be effectively tested in minimal media supplemented with food bacteria. The effects of the organic medium on the detection of toxicity by contaminants other than metals remains to be determined, but there were clear effects of metal binding and reduced metal availability for cadmium and copper tested in organic media.

Considerable interest exists in reducing the time and complexity of toxicity estimation in routine screening of contaminants and contaminant mixtures in waste discharges and at sites impacted by hazardous materials (Blaise 1991). Sometimes, only limited information is available for contaminant effects on non-mammalian species. Rapid tests that are logistically simple provide an important element in the hazard assessment process. Short-term tests using organisms that require minimal culturing are presently limited to tests such as those using the bacterium *Photobacterium phosphoreum* (Microtox), the ciliate *Colpidium*

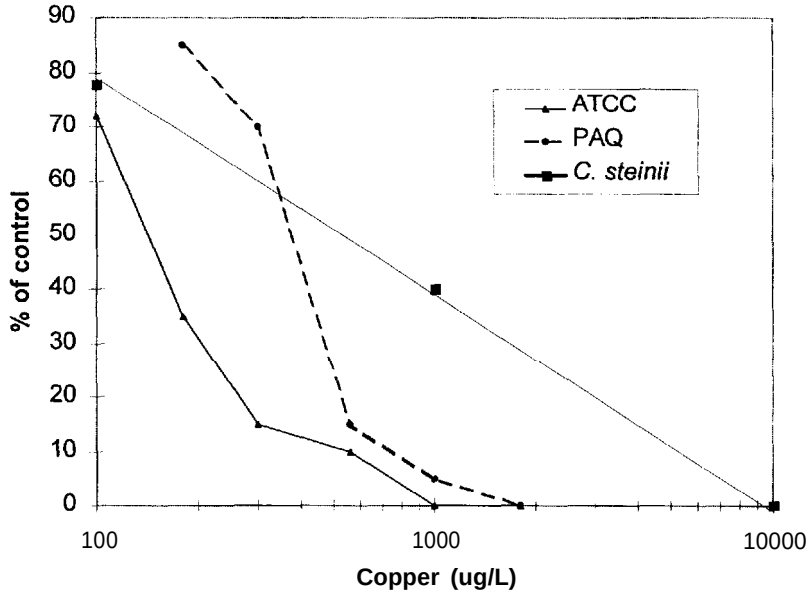
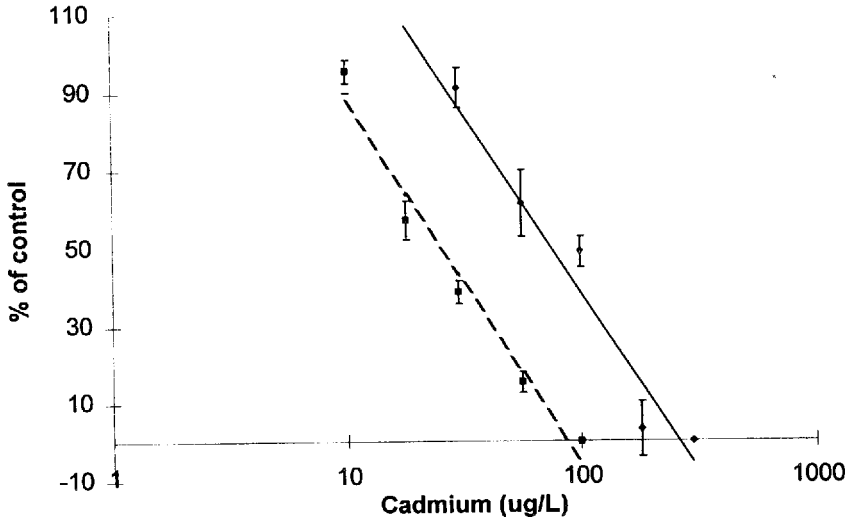



**Figure 1.** Results of preliminary experiments examining sensitivity of strains of *Colpoda* to copper. ATCC = American Type Culture Collection (ATCC 30917), PAQ = strain isolated from local soils, *C. steinii* = results reported by Forge et al. (1993) for C. *steinii.*

*campylum* (Dive et al. 1990), and the rotifer *Branchionus calcyfloris.* In each case, the organisms are cultivated over a short term from either lyophilized material or resting stages (cysts, eggs). Increasing the number and diversity of these model species is essential to provide a selection of test species for "battery of tests" approaches to assessing hazard. Each test species has different environmental requirements and can provide useful information on effects in different environmental compartments.

In addition to the studies reported here, we have examined the effects of the three toxic metals on the growth of *Colpoda inflata* from cysts. Experiments similar to those reported above were begun with 100 cysts/ml in 10% Sonneborn's medium. Excystment and growth proceeded for 48 hr. These experiments showed an increase in sensitivity to the toxicant but an undesirable increase in the size of confidence intervals as a result of increased variability among replicates. For example, tests with cysts gave an estimated IG50 for copper of 140 ug/L (20-350 ug/L, 95% confidence interval) compared to 575 ug/L (573-579 ug/L) in the organic medium and 30 ug/L (27-69 ug/L) in the minimal salts medium. For zinc toxicity, IG50 estimates were 250 ug/L (87-530 ug/L) for cysts compared to 161 ug/L (150-171 ug/L) in organic medium and 119 ug/L (95-151 ug/L) in the



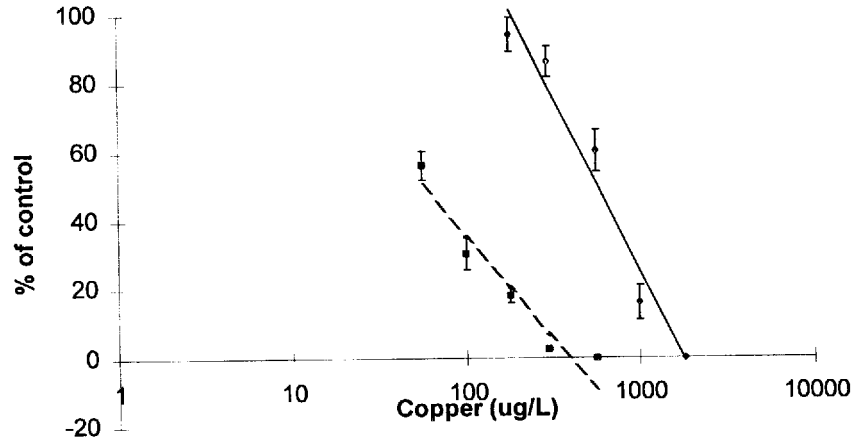




**Figure 2.** Effects of organic media on cadmium (top) and copper (bottom) toxicity. The fitted lines are for tests conducted with different test media: 10% Sonneborn's medium (solid line) and minimal salts medium (dashed line).

Table 1. Effects of toxic metals on growth of *Colpoda inflata* cells and cysts in a single test series. All values are IG50 in ug/L. Values in parentheses are 95% confidence intervals based on inverse prediction. Acute range based on water quality criteria (USEPA 1986).

| Metal | 10% Sonneborn's IG50 | 5% Sonneborn's IG50 | Minimal Salts IG50 | Acute range |
|---|---|---|---|---|
| Cadmium | 75 (74-76) | 28 (26-30) | 25 (23-28) | 1 - 28 000 |
| Copper | 575 (527-583) | 148 (142-153) | 30 (27-69) | 17 - 10 200 |
| Zinc | 161 (150-171) | 72 (63-80) | 119 (95-151) | 51 - 88 900 |

minimal salts medium. It was not possible to achieve reliable excystment and growth for experiments with cysts begun in the minimal salts medium. Therefore, initiating tests with cysts cannot be recommended.

Our studies have focused on a ciliated protist found in both freshwater and in soil water. Additional models for rapid, microscale tests are needed for other protists such as chromophyte flagellates and for additional multicellular species. Requirements for culturing of test species places severe constraints on the timing and locations at which testing can be done. Tests that minimize culturing effort can expand the range of circumstances over which toxicity assessment can be done, allowing limited resources to be spent on environmental assessment, not culturing.

*Acknowledgements.* This work was supported, in part, by the US Army Medical Research and Development Command through grant DAMD17-95-1-5068. The assistance of Caroline Belback, Roxy Grossnickle, Michael Hummel and Ian Harrell is gratefully acknowledged.

## REFERENCES

Berk S, Gunderson JH, Derk LA (1985) Effects of cadmium and copper on chemotaxis of marine and freshwater ciliates. Bull Environ Contam Toxicol 27:577-582

Blaise C (1991) Microbiotests in aquatic ecotoxicology: characteristics, utility, and prospects. Environ Toxicol Water Qual 6:145-155
Dive D, Blaise C, Robert S, Le Du A, Bermingham N, Cardin R, Kwan A, Legault R, MacCarthy L, Moul D, Vielleux L (1990) Canadian workshop on the *Colpidium campylum* ciliate growth inhibition test. Z angew Zool 77:49-64
Dunnett CW(1955) Multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 50:1096-1121
Foissner W, Blatterer H, Berger H, Kohmann F (1991) Taxonomische und ökologisches Revision der Ciliaten des Saprobiensystem, Band I: Cyrtophorida, Oligotrichida, Hydrotrichida, Colpodea. Informationsnberichte der Bayerische Landesamt für Wasserwirtschaft, München
Forbes VE, Forbes TL (1994) Ecotoxicology in theory and practice. Chapman and Hall, London
Forge TA, Berrow ML, Darbyshire JF, Warren A (1993) Protozoan bioassays of soil amended with sewage sludge and heavy metals using the common soil ciliate *Colpoda steinii*. Biol Fertil Soils 16:282-286
Hart KM, Cairns J Jr (1984) The maintenance of structural integrity in freshwater protozoan communities under stress. Hydrobiologia 108:171-180
Roberts RO, Berk SG (1990) Development of a protozoan chemoattraction bioassay for evaluating toxicity of aquatic pollutants. Toxicity Assess 5:279-292
Ruthven JA, Cairns J Jr (1972) The response of freshwater protozoan artificial communities to metals. J Protozool 20:127-135
Schultz TW, Bryant SE, Lin DT (1994) Structure-toxicity relationships for *Tetrahymena:* aliphatic aldehydes. Bull Environ Contam Toxicol 52:279-285
Sokal RR, Rohlf FJ (1981) Biometry, 2nd ed, WC Saunders Publishers, New York
USEPA (1986) Quality criteria for water. Office of Water, Washington, DC