Bull. Environ. Contam. Toxicol. (1991) 47:302–307


Bulletin of Environmental Contamination and Toxicology

# Planarians in Toxicology. Responses of Asexual *Dugesia dorotocephala* to Selected Metals

Moses M. Kapu and David J. Schaeffer

Department of Veterinary Biosciences, University of Illinois, 2001 South Lincoln Avenue, Urbana, Illinois 61801, USA

The planarian *Dugesia dorotocephala* is a freshwater invertebrate found in unpolluted flowing surface waters. Planarians have a sensitive nervous system with synapses and a true brain (Best and Noel 1969) and evidence these in a variety of social and response behaviors (McConnell 1967). The inclusion of planarians in a screening battery would provide improved sensitivity in detecting toxicity because planarians commonly respond to lower levels of contamination than do other species (Burnham 1981; Barndt and Bohn 1985). Furthermore, planarians, but not daphnids, can be included in a higher tier since they may exhibit a multiplicity of complex responses including inhibition and stimulation of reproduction, teratogenesis and tumorigenesis (Best and Morita 1982; Schaeffer et al. 1991a).

Numerous toxicity tests have been conducted to determine the acute and chronic effects of toxicants to provide data necessary for the development of water quality criteria. However, water quality standards are generally based on single substance acute (48-96 hr) and subchronic/chronic (weeks to months) tests, and few studies have examined the toxicity of mixtures of pollutants using constituents at their regulated levels (Spehar and Fiandt 1986).

The appropriateness of Illinois water quality standards for metals was investigated using a 1-hr behavioral test based on the responses of the planarian *D. dorotocephala*. One possible difficulty with water quality standards for metals is that the standard for each metal is usually established without regard to the effects of other metals present in the receiving water.

## MATERIALS AND METHODS

Asexual *D. dorotocephala* (18 to 20 mm; Carolina Biological Supply, Gladstone, Oregon) were maintained on an 8:16 hr light:dark cycle in medium containing 300 mg/L $CaCl_2$, 120 mg/L $MgSO_4$, 60 mg/L $NaHCO_3$, and 6 mg/L KCl; hardness = 370 mg-equivalent $CaCO_3$/L (Kostelecky et al. 1989). Animals were fed 24-48 hr prior to the start of testing. Tests of metals were carried out at the 1987 Illinois water quality standards concentrations of: B, Fe, Ni, Se, and Zn = 1.0 mg/L; Cr(VI) = 0.05 mg/L; Cu = 0.02 mg/L; Pb = 0.1 mg/L. Some tests used levels 10 to 50 times higher. Mixtures containing each of these metals at 0.5, 1, 2, and 10 times the criteria levels were also

Address correspondence to Dr. David Schaeffer.

tested. The pH was adjusted to 6 to 8 for these tests. Metal solutions were prepared in medium. Twenty animals were used at each test concentration. Independent duplicate trials were carried out.

Behavioral responses were recorded at 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, and 60 min. Several distinct responses were scored, and the following were most frequently observed: (1) restlessness, (2) hyperkinesia, (3) spiraling, (4) head/nose twist, (5) shape change, and (6) labored movement. Animals often simultaneously showed several responses. Results were also evaluated using a modification of a scoring system devised for environmental samples using phenol as the reference toxicant (Grebe and Schaeffer 1991). Scores were evaluated relative to all the samples in the group. Data from other metals and organic compounds (Schaeffer et al. 1991b) provided an additional basis for evaluation. The scores used were:

> Weak: A few animals show shape changes, nervousness, head/nose twist, or labored movement. The toxicity pattern was similar to that of animals to 32.6 mg/L phenol.
>
> Moderate: Most animals are restless, and most show a variety of movement and shape-related responses. The pattern of toxic responses in these samples is similar to that produced by 65.3 mg/L phenol.
>
> Strong: Most animals go through cycles of restlessness or hyperkinesia, labored movement increases with time and show concurrent shape changes, depression is evident, and some animals become morbid. In some instances, animals removed to clean medium die within 48 hours. Toxicity patterns are similar to exposure to 65.3 to 97.9 mg/L phenol.

To facilitate statistical analysis and interpretation, responses were grouped into 4 categories and 3 time groups: response category I = restlessness and hyperkinesia, II = spiraling and head/nose twist, III = other shape change, and IV = labored movement (Tables 1, 4) or relative to phenol (Table 3). Although other behavioral responses were found, these were observed only occasionally and were not considered in the analysis. These responses are from a response checklist for asexual *D. dorotocephala* (Grebe and Schaeffer 1991). A similar checklist of coded behavior and toxic signs is used for the fathead minnow (*Pimephales promelas*; Geiger et al. 1988).

The effects of concentration and time on the patterns of responses were determined using $X^2$ analysis with linear contrasts (Sauer and Williams 1989) as implemented in CONTRAST (Hines and Sauer 1989). Concentration effects were tested using count data transformed into failure rates (proportions) by dividing each count by the sum of all the counts. Temporal changes within a concentration were determined from failure rates obtained using the total number of counts for that concentration as the divisor.

## RESULTS AND DISCUSSION

With the exception of Fe(III) ($P > 0.05$), the patterns of response for an individual metal significantly changed with time ($P < 0.05$) (Table 1). Generally, more animals showed classes III and IV responses as the duration of exposure increased. This suggests a concentration-response effect of the individual metal. Iron(II) is believed to be more easily absorbed across biological .awoff

Table 1. Behavioral responses in 20 planarians/concentration exposed to metals in medium of hardness of 370 mg-equivalent $CaCO_3$/L.

| Metals[b] | 1 to 5 min | | | | | 10 to 30 min | | | | | 40 to 60 min | | | | | P[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I[a] | II | III | IV | P[d] | I | II | III | IV | P[d] | I | II | III | IV | P[d] | |
| B (1) | 61 | 46 | 0 | 0 | * | 41 | 20 | 3 | 3 | - | 9 | 16 | 24 | 23 | - | * |
| B (10) | 72 | 66 | 1 | 0 | | 32 | 30 | 2 | 1 | | 14 | 26 | 9 | 8 | | * |
| $Cr^{6+}$ (0.05) | 58 | 27 | 0 | 0 | * | 39 | 18 | 4 | 0 | * | 27 | 12 | 17 | 22 | - | - |
| $Cr^{6+}$ (0.5) | 110 | 49 | 17 | 0 | | 14 | 23 | 5 | 0 | | 29 | 16 | 26 | 23 | | * |
| Cu (0.02) | 87 | 37 | 6 | 0 | * | 44 | 31 | 10 | 10 | - | 11 | 11 | 41 | 24 | - | * |
| Cu (0.2) | 105 | 38 | 26 | 0 | | 39 | 23 | 28 | 6 | | 12 | 7 | 49 | 44 | | * |
| $Fe^{3+}$ (1) | 115 | 66 | 0 | 0 | * | 41 | 23 | 5 | 0 | * | 40 | 17 | 4 | 0 | - | * |
| $Fe^{3+}$ (10) | 35 | 13 | 0 | 0 | | 17 | 15 | 0 | 0 | | 85 | 34 | 14 | 4 | | * |
| $Fe^{3+}$ (50) | 98 | 77 | 17 | 0 | | 45 | 12 | 2 | 0 | | 29 | 12 | 12 | 14 | | * |
| Ni (1) | 10 | 22 | 0 | 0 | | 20 | 13 | 5 | 0 | * | 18 | 21 | 1 | 9 | * | - |
| Ni (10) | 13 | 64 | 9 | 0 | * | 24 | 15 | 3 | 0 | | 20 | 16 | 4 | 5 | | * |
| Ni (50) | 112 | 59 | 10 | 0 | | 52 | 5 | 36 | 17 | | 33 | 7 | 60 | 38 | | * |
| Pb (0.1) | 87 | 9 | 10 | 5 | * | 37 | 8 | 6 | 6 | - | 16 | 10 | 19 | 20 | * | * |
| Pb (1.0) | 113 | 17 | 16 | 5 | | 37 | 17 | 9 | 9 | | 18 | 7 | 35 | 33 | | * |
| Se (1) | 15 | 37 | 0 | 0 | * | 10 | 27 | 7 | 7 | * | 10 | 7 | 29 | 34 | - | * |
| Se (10) | 73 | 46 | 0 | 0 | | 38 | 28 | 4 | 4 | | 23 | 13 | 19 | 21 | | * |
| Zn (1) | 13 | 23 | 4 | 0 | * | 9 | 10 | 1 | 0 | * | 7 | 16 | 1 | 7 | * | * |
| Zn (10)[c] | 131 | 91 | 73 | 0 | | 45 | 41 | 43 | 23 | | 11 | 4 | 48 | 36 | | * |

[a]Roman numerals I through IV represent clusters of responses. See text.
[b]Numbers in parenthesis represent concentrations of metals in mg/L. Overall concentration effects (i.e., between rows) were significant at $P < 0.05$ for each metal.
[c]40% mortality observed after 1 hr and 50% mortality after < 24 hr.
[d]Means between concentrations within times differ at $P < 0.05$ (*).
[e]Means for each time group within the concentration differ at $P < 0.05$ (*).

membranes than iron(III). This is true for the absorption of dietary iron through the mucosae in the small intestine. And since the animals were fasting, the uptake of fluid and solutes were mainly by cutaneous diffusion, so it is possible that the infiltration of Fe(III) ions may have been inhibited across the planarian skin in a similar manner. However, studies using other compounds of iron would be necessary to confirm this.

Each metal produced behavioral toxicity at its criterion concentration, and had overall response patterns which differed significantly ($P < 0.05$) with concentration. Depending on the metal, responses at different concentrations differed for all, or only some, time periods (Tables 1-3). For example, B and Cu concentrations differed only at 1-5 min whereas Ni and Zn concentrations differed in each time period. For each metal, both the absolute number of responses, and concentration differences, tended to diminish with time (Table 2). As judged by the total number of responses, nickel at 1-10 mg/L and zinc at 1 mg/L were the least toxic metals, but were the most toxic at 50 and 10 mg/L, respectively. Water hardness can affect the toxicity of Cu, Pb, and Zn (Spehar and Fiandt 1986; USEPA 1986), so the hardness of the medium may account for some of the observed toxicity differences between metals.

Table 2. Total responses by time period and concentration for n = 20 planarians at each concentration.

| Metal | Conc. mg/L | 1-5 min | 10-30 min | 40-60 min | Total |
|---|---|---|---|---|---|
| B | 1 | 107 | 67 | 72 | 246 |
| B | 10 | 139 | 65 | 57 | 261 |
| Cr(VI) | 0.05 | 85 | 61 | 78 | 224 |
| Cr(VI) | 0.50 | 176 | 42 | 94 | 312 |
| Cu | 0.02 | 130 | 95 | 87 | 312 |
| Cu | 0.20 | 169 | 96 | 112 | 377 |
| Fe | 1 | 181 | 69 | 61 | 311 |
| Fe | 10 | 48 | 32 | 137 | 217 |
| Fe | 50 | 192 | 59 | 67 | 318 |
| Ni | 1 | 32 | 38 | 49 | 119 |
| Ni | 10 | 86 | 42 | 45 | 173 |
| Ni | 50 | 181 | 110 | 138 | 429 |
| Pb | 0.10 | 111 | 57 | 65 | 233 |
| Pb | 1.0 | 151 | 72 | 93 | 316 |
| Se | 1 | 52 | 51 | 80 | 183 |
| Se | 10 | 119 | 74 | 76 | 269 |
| Zn | 1 | 40 | 20 | 31 | 91 |
| Zn | 10 | 295 | 152 | 99 | 546 |

Table 3. Phenol-equivalent toxicity rating of regulated metals (mg/L) and mixtures at 0.5x, 1x, 10x and 50x criterion level.

| Metal | Weak | Moderate | Strong |
|---|---|---|---|
| Cu | --- | --- | 0.02, 0.2 |
| Cr(VI) | --- | 0.05 | 0.5 |
| Fe(III) | 1 | 10, 50 | --- |
| Ni | 1, 10 | 10 | 50 |
| Pb | --- | --- | 0.1, 1.0 |
| Se | --- | 1, 10 | --- |
| Zn | 1 | --- | 10 |
| Mixture | 0.5x | 1x, 2x | 10x |

Mean overall response rates for the mixtures of 0.0240, 0.0450, 0.0435, 0.0542 responses/min, differed significantly between concentrations (0.5x, 1x, 2x, 10x criteria, respectively). With the exception of a nonsignificant difference between 1x and 2x, the severity of the responses increased significantly with concentration. Time periods I and III, but not II, differed between concentrations. Within each concentration except 2x, temporal effects were significant ($P < 0.05$).

Table 4: Behavioral responses in groups of 20 planarians exposed to metal mixtures at multiples of Illinois criterion levels (mg/L).

| | Observation Time (Min) | | | | | |
|---|---|---|---|---|---|---|
| | 1 to 5 | | 10 to 30 | | 40 to 60 | |
| Mixture / Response[a] | I | II | I | II | I | II |
| 0.5x Criterion | 7 | 12 | 15 | 24 | 14 | 24 |
| Criterion | 27 | 51 | 21 | 35 | 18 | 28 |
| 2x Criterion | 30 | 22 | 26 | 29 | 27 | 40 |
| 10x Criterion | 62 | 41 | 27 | 19 | 33 | 35 |

[a]Response I = restlessness and hyperkinesia
Response II = spiraling and head/nose twist

The mixtures were separately compared with the integer-rounded mean counts for the individual metals at their criteria levels. For the 1x criterion mixture, the overall response rates for these two groups, and for period III differed marginally ($P < 0.07$), but did not differ for periods I and II ($P >> 0.10$). However, the overall temporal subgroup response rates for the 0.5x mixture were less than the rates for the individual metals ($P < 0.05$). The overall response rate for the 2x mixture was significantly smaller than the rate for the individual metals ($P < 0.05$) and a significant temporal difference existed for period I ($P < 0.05$). The overall response rate for the 10x mixture, and for each subgroup, did not differ from the rates for the mean individual metals.

Toxicity generally increased with concentration and with exposure time.

These increases suggest that there is a potential for the metals, alone or in combination, to cause deleterious effects in exposed organisms at concentrations below present proposed single-chemical water criteria (USEPA 1986). Further study is therefore necessary to evaluate the interaction of chemical compounds and determine the effects of these on sensitive organisms. The rapid test used here provides a convenient bioassay for rapid, preliminary evaluation of water toxicity.

## REFERENCES


Barndt G, Bohn B (1985) Hilfen zur biologischen und chemischen gutebestimmunq von fliessgewassern. PZ85-07-0021. Padagogisches Zentrum, West Berlin

Best JB, Noel J (1969) Complex synaptic configurations in planarian brain. Science 164:1070-1071

Best JB, Morita M (1982) Planarians as a model system for in vitro teratogenesis studies. Teratog Carcinog Mutagen 2:277-292

Burnham L (1981) *Daphnia magna*--Bioassay species for the toxicity of in situ shale oil process wastewater. Thesis submitted to Microbiology Department, Colorado State University, Fort Collins, Colorado

Geiger DL, Call DJ, Brooke LT (1988) Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). IV. Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, pp 15-18

Grebe E, Schaeffer DJ (1991) Planarians in toxicology. Standardization of a rapid neurobehavioral toxicity test using phenol in a crossover study. Bull Environ Contam Toxicol (in press)

Hines JE, Sauer JR (1989) Program CONTRAST. A general program for the analysis of survival or recovery rate estimates. Fish Wildl Tech Rep 24. U.S. Fish and Wildlife Service, Washington, D.C.

Kostelecky J, Elliott B, Schaeffer DJ (1989) Planarians in Toxicology. I. Physiology of sexual-only *Dugesia dorotocephala*. Effects of diet and population density on adult weight and cocoon formation. Ecotox Environ Saf 18:286-295.

McConnell JV (1967) A manual of psychological experimentation on planarians, 2nd ed. University of Michigan Press, Ann Arbor, Michigan

Sauer JR, Williams BK (1989) Generalized procedures for testing hypotheses about survival or recovery rates. J Wildl Manage 53:137-142

Schaeffer DJ, Johnson L, Dean-Colomb W, Gills R, Grebe E, Hankenson K, Hansen L, Kapu M, McLaughlin G, Ness D, Schaeffer S. (1991a) Planarians in toxicology. VIII. Bioassay for environmental carcinogens. In: Munawar M (ed) Proceedings of the international symposium on aquatic ecosystem health, University of Waterloo, Ontario, Canada, July 23-26, 1990. Kluwer Academic Publishers, The Netherlands, in press

Schaeffer DJ, Goehner M, Grebe E, Hansen LG, Hankenson K, Herricks EE, Matheus G, Miz A, Reddy R, Trommater K (1991b) Evaluation of the "Reference Toxicant" addition procedure for testing the toxicity of environmental samples. Bull Environ Contam Toxicol (in press)

Spehar RL, Fiandt J-T (1986) Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ Toxicol Chem 5:917-931

USEPA (1986) Quality criteria for water 1986. EPA-440/5-86-001. U.S. Environmental Protection Agency, Washington, D.C.


Received July 23, 1990; accepted January 29, 1991.