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Neurobehavioral Toxicity of Cadmium Sulfate to the Planarian *Dugesia dorotocephala*

Eric Grebe and David J. Schaeffer

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

We are developing bioassays which use planarians (free-living platyhelminthes) for the rapid determination of various types of toxicity, including acute mortality, tumorigenicity, and short-term neurobehavioral responses. Our motivation for using these animals is due to their importance as components of the aquatic ecology of unpolluted streams (Hyman 1951; Kenk 1976), their sensitivity to low concentrations of environmental toxicants (Kostelecky 1988; Kolkwitz and Marson 1908; Kenk 1976; Steinmann and Breslau 1913), and the presence of a sensitive neurological system (McConnell 1967) with a true brain (Best and Noel 1969) which allows for complex social behavior (McConnell 1967). A previous paper described the results of a neurobehavioral bioassay using phenol in a crossover study (Grebe and Schaeffer 1991). This paper reports a similar crossover study using cadmium sulfate.

MATERIALS AND METHODS

Dugesia dorotocephala (Carolina Biological Supply, Gladstone, Oregon) were maintained at 19 C in synthetic media (Kostelecky et al. 1989). Animals were fed once-weekly for 3 h on beef liver and then placed in fresh media. Following the methods of Grebe and Schaeffer (1991), crossover tests in a 4 x 4 Latin square were carried out at room temperature (21-22 C) by exposing groups of 25 planarians to 25.4, 50.9, 76.3 or 101.8 mg/L of Cd(II) (from $3CdSO_4.8H_2O$; J. T. Baker Chemical Co., Phillipsburg, New Jersey) in media (pH $\approx$ 7.2). Neurobehavioral responses (Table 1 in Grebe and Schaeffer 1991) were recorded at 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, and 60 min of exposure. Animals were maintained for 48 h in clean media between exposures.

Each neurobehavioral response was analyzed as a Latin square with a carryover term (Cochran and Cox 1957) and observation time as a covariate. The principle analysis of toxicity combined separate responses into the categories of **Loc**omotive [(1) restlessness, (2) hyperkinesia, (3) swims upside down], **Morph**ological [(4) spiraling, (5) head/nose twist, (6) shape change, (7) ornamentation, (8) banana curl or coil], **Neuro**logical [(9) convulsions, (10) nervous signs], **Morb**idity [(11) labored movement, (12) depression, (13) unconsciousness, (14) death], and **Prot**ective [(15) pharynx protrusion, (16) vomiting, (17) mucus covering body, (18) lesions]. Times were pooled into the three periods of 1-5 min, 10-30 min and 40-60 min. Stepwise linear regression

Send reprint requests to Dr. David Schaeffer at the above address.

was used to develop the relationship between the cumulative number of responses for a category and the observation time and concentration.

RESULTS AND DISCUSSION

Total responses for the four exposure groups are given in Table 1 for each response category by concentration and time. No responses in the protective category were observed.

Table 1. Total Responses[1] to Cadmium(II)

Conc., mg/L	Time, min	Locomotive	Morphologic	Neurologic	Morbidity
25.4	1-5	30	27	4	56
25.4	10-30	7	19	4	118
25.4	40-60	1	9	4	83
	Total	38	55	12	157
50.9	1-5	105	85	5	21
50.9	10-30	29	40	8	40
50.9	40-60	11	64	2	100
	Total	145	189	15	161
76.3	1-5	136	162	58	19
76.3	10-30	12	25	8	41
76.3	40-60	5	135	0	185
	Total	153	322	66	245
101.8	1-5	151	122	93	2
101.8	10-30	42	36	15	24
101.8	40-60	15	126	6	204
	Total	208	284	114	230

[1]**Loc**omotive (restlessness, hyperkinesia, swims upside down), **Morph**ological (spiraling, head/ /nose twist, shape change, ornamentation, banana curl or coil), **Neuro**logical (convulsions, nervous signs), **Morb**idity (labored movement, depression, unconsciousness, death)

For spiraling and labored movement, the carryover term for the Latin square analysis was statistically significant ($P < 0.05$). In a repeat experiment, the carryover term was statistically significant ($P < 0.5$) for spiraling but not for labored movement. These results suggest that either the 48 h washout period between exposures was insufficient or that a persisting biochemical change had occurred, even at the lowest concentration tested. Mortality data from our tumor experiments using 0.13 mg/L Cd(II) are consistent with the latter.

Stepwise linear regression was carried out for each response category using concentration (1X, 2X, 3X and 4X 25 mg/L Cd(II)), time period (1, 2, 3), and concentration x time period as regressors. In each model, the intercept was not statistically significant ($P > 0.05$) and was omitted. Table 1 shows that all of the concentrations tested produced morbidity responses. The models in Table 2 show that morbidity increases were related to time period but not concentration. In contrast, morphological responses increased with concentration but not time period. The total numbers of locomotive and neurological responses increased with concentration at all times, although the total

numbers of these responses rapidly declined with time >5 min.

Table 2. Results of Stepwise Regression for Cd(II) Toxicity

Response	Conc., mg/L	Time, min	Conc. x Time
Locomotive	46.6	---	-13.1
Morphologic	28.2	---	---
Neurologic	20.7	---	-5.97
Morbidity	---	39.1	----

Qualitative insights about these responses were obtained by comparing the models for Cd(II) (Table 2) with those for phenol (Table 3; from Grebe and Schaeffer 1991). To determine whether numerical factors were primarily causing the selection of different variables in the models, the counts at each time period/concentration were separately ranked for each compound. Linear regressions on the ranks resulted in the same selections of variables.

Table 3. Results of Stepwise Regression for Phenol Toxicity

Response	Conc., mg/L	Time, min	Conc. x Time
Locomotive	21.3	---	-7.3
Morphologic	---	-12.6	10.7
Neurologic	3.6	-1.5	-0.59
Morbidity	---	---	5.8

For both toxicants, the locomotive response models increased with concentration and asymptotically over time. The models for neurological responses also were similar although time was more important for the phenol than for the Cd(II) model. The models for morphometry and morbidity differed qualitatively for Cd(II) and phenol. Morphometry responses for Cd(II) were only related to concentration whereas for phenol they were primarily related to time. A generalized χ^2 test (Sauer and Williams 1989; Hines and Sauer 1989) showed that the relative frequencies for morphology (responses 3, 4, 7) for phenol (7.7%, 24.5%, 67.7%) and Cd(II) (16.9%, 41.5%, 41.6%) differed significantly ($P < 0.05$).

For each concentration of Cd(II), the total number of responses in each time period, when viewed across time periods, is "U"-shaped. This pattern is due to differences in the proportional representation of each response category in each time period. Generally, time period 1 comprised mainly locomotive responses, period 3 morbidity responses, and period 2, a transitional period, had these categories about equally represented. If only the morphological responses are considered, a "U"-shaped pattern across time was found for each concentration above 1 mg/L. This category was mostly (4) spiraling during period 1 and mostly (6) shape change during period 3. For phenol concentrations of 22-64 mg/L, while the same temporal relationship existed between the locomotive and morbidity responses as found for Cd(II), the morphological responses increased approximately linearly over time and were in higher proportion of total responses.

It is well known that the mechanisms of acute toxicity differ for phenol and cadmium. At this time we have no information on phenol or Cd(II) metabo-

lism by *D. dorotocephala*. However, we have found that β-glucosidase and/or β-glucuronidase activities specifically declined after 1-, 7- and 13-day *in vivo* exposures to Cd(II). In mammals, Cd(II) is transported in blood bound to red blood cells and to large-molecular-weight plasma proteins such as albumin. Inorganic cadmium accumulates in the kidney and is detoxified through gastrointestinal absorption. In contrast, phenol is a general protoplasmic poison which enters into a loose combination with protein and which causes anesthesia due to the death of nerve endings. Fish exposed to phenol rapidly exhibit behavioral responses (Babich and Davis 1981) similar to those for planarians. Phenol is detoxified in mammals by oxidation, conjugation and excretion through the kidneys. An intriguing aspect of our results with *D. dorotocephala* is that qualitative and quantitative differences in the mechanisms of toxicity were discernible in the behavioral changes occurring during a 1 h exposure.

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