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Evaluation of the Sensitivity of Rapid Toxicity Tests Relative to Daphnid Acute Lethality Tests

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Acute bioassays are well developed and widely used test methods for toxicity assessment of chemicals and effluents. Although these tests are robust and typically provide very definitive results, they can be very time consuming, some requiring 48 to 96 hours to complete. New and revised commercial test methods are being developed, many of these with the goal of increased test rapidity. Some of these tests have as endpoints measurements of enzymatic activity or amount of bioluminescence instead of mortality. Presently, many tests are available that take 24 hours or less to complete. We compared several of these newer commercial tests with the standard 48-hour *Ceriodaphnia dubia* mortality test. One of our main interests in these tests is as a tool for adding toxicity data to biological field surveys of aquatic systems. The ability to perform tests in a motel room, field office, or other remote location in a short time would make it much easier to make toxicity information part of the field survey package.

Tests that we used were those that could be obtained "off the shelf' from commercial vendors and included: a 1-hour enzymatic inhibition test using the daphnid *C. dubia;* a three-hour enzymatic inhibition microplate assay with the bacteria *Escherichia coli;* and a 24-hour mortality test with the rotifer, *Brachionus calyciflorus.* We also obtained toxicity data for Microtox®, a microbial test, from the literature for comparison with the above tests. Mortality results from 24-hour *C. dubia* tests are also reported. Our objective was to rank the sensitivity of these "rapid tests" compared with the standard 48-hour *C. dubia* mortality test.

MATERIALS AND METHODS

Six chemicals were tested: zinc (as ZnCl); copper (as $CuSO_4 \bullet 5\ H_2O$); cadmium (as $Cd\ (NO_3)_2 \bullet 4\ H_2O$); Malathion; 2,4-dichlorophenoxyacetic acid (2,4-D); and dipotassium salt of Endothall. Chemical sources included Cheminova (Malathion), Elf Atochem (Endothall), EM Science (cadmium, zinc), DowElanco (2,4-D), and VWR Scientific (copper). Chemicals tested were those thought

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likely to impact Bureau of Reclamation projects in areas of mine drainage or agriculture returns or drains. Stock working solution concentrations were measured using inductively coupled plasma/emission spectroscopy for metals and gas chromatography/mass spectroscopy methods for organics. A multiparameter probe and meter were used in measurement of physicochemical parameters, and hardness was determined with titration.

Working solutions and dilutions were made in reconstituted moderately-hard water (Peltier and Weber 1985). Adjustments were made to pH only for working solutions of 2,4-D because pH values were <4.0. All other working solution pH's were ≥7.3. No carrier solvents were used.

The IQ Toxicity Test™ was performed with 4-day-old *Ceriodaphnia dubia* using techniques similar to those presented in Janssen *et al.* (1993). Daphnids were not fed for at least six hours before testing. Daphnids were exposed to triplicate toxicant dilutions (six *C. dubia* per 5 ml of solution) for one hour, when an enzyme substrate (4-methylumbelliferyl-β-D-galactoside) was added for ingestion by the animals. After 15 minutes incubation, organisms were exposed to ultraviolet (U.V.) light and number of fluorescent daphnids counted. Cleavage or splitting of the substrate results in fluorescence under U.V. light and suggests that the animal is unaffected.

The MetPLATE™ kit was performed according to methodology in Bitton *et al.* (1994). This test uses freeze-dried *Escherichia coli* bacteria as the test organism. A bacterial suspension was incubated with toxicant dilutions in a 96-well microplate. Enzyme chromogenic substrate (reacts with β-galactosidase) was added and the plate was incubated for 2-3 hours at 35°C for color development. Absorbance was measured at 570 nm using a microplate reader (BIO-TEK Instruments, Inc., ELX800). A reduction in intensity of the purple color, compared with controls, shows toxicity. All tests were performed in triplicate.

The rotifer test was performed following the method of Snell *et al.* (1991) using *Brachionus calyciflorus.* Test animals were hatched from cysts in the laboratory. Ten rotifers were used in each treatment and controls, with three 1 -ml replicates of each concentration. Well plates containing solutions and rotifers were held in the dark at 25°C, and observed for mortality after 24 hours.

Mean 5-minute EC50 values (dose that produces a 50% decrease in a sublethal response), ranges, and the number of tests for the different chemicals were calculated and collected from a variety of literature sources. The Microtox® assay is based on the inhibition of light production of the bioluminescent microbe, *Vibrio fischeri* in the presence of toxicants. A photometer is used to provide temperature control and measurement of light emission.

Acute 48-hour toxicity tests (EPA 1991) were conducted using *C. dubia* (< 24-hr

old). Five *C. dubia* were used at each of four replicate concentrations containing 10 mls of solution. Tests were incubated at 25°C with a photoperiod of 1 g-hour light:6-hour dark. Organisms were fed during testing. Results of the 48-hour tests were used as a benchmark against which other tests were compared.

LC50 (dose lethal to 50% of the test organisms) and EC50 values were calculated for tests by the trimmed Spearman-Karber method (Hamilton *et al.* 1977) except for values from MetPLATE™ kits. Because these test results are measured as absorbance, the degree of inhibition was determined, considering the control to represent 0% inhibition. Data were plotted as percent inhibition vs. log final toxicant concentration and the EC50 (50% inhibition) determined from linear regression analysis of the data.

To compare sensitivity of tests they were ranked from 1 to 100. The most sensitive test result for each compound was assigned the lowest number and the least sensitive test result the highest number (*sensu* Toussaint *et al.* 1995). Other tests were ranked within this 1 to 100 range about the LC50 or EC50 obtained for the test. Ranks were then averaged for each test to yield a mean rank across all compounds. When an LC50 could not be calculated, the test with the highest "greater than" value was ranked highest. Although rankings could not be analyzed statistically, this method allows for a graphical comparison of relative sensitivity.

RESULTS AND DISCUSSION

Toxicity test results are in Table 1. Mean, minimum, and maximum physicochemical parameters for dilution water (n=l6) used in tests were: conductivity=283, 267-299µS/cm; dissolved oxygen (D.O.)=6.9, 5.8-9.0 mg/L; pH=8.32, 8.05-8.54; and hardness=81, 77-87 mg/L. Values measured for working solutions of toxicants (n=20) were: conductivity=295, 264-457 µS/cm; D.O.=7.2, 5.8-10.0 mg/L; and pH=7.74, 7.30-8.31.

The IQ Toxicity Test'" generally were within an order of magnitude of the standard 48-hour test values (Table 1). This test had an advantage over the other enzyme assay test because although β-galactosidase is relatively insensitive to organics (Peterson and Stauber 1996), test organisms were sensitive. In several cases, organisms died in the higher concentrations and, as a result, did not fluoresce. One difficulty with this test, however, is that the end point is not definitive. There may be a range of fluorescence and although control daphnids are tested for comparison, deciding whether a particular daphnid is affected can be difficult.

EC50 values from metals tested with the MetPLATE" kit were similar to the 48-hour standard *Ceriodaphnia dubia* test and much lower than those reported for the

Table 1. LC50/EC50 end point of tests (µg/L). Sensitivity ranks are in **bold.**

Compound	Test Method					
	IQ Toxicity Test™	MetPLATE™	Rotifer	24-hr *C. dubia*	48-hr *C. dubia*	Microtox®
	EC50 and 95% confidence interval	EC50 and 95% confidence interval	LC50 and 95% confidence interval	LC50 and 95% confidence interval	LC50 and 95% confidence interval	Mean EC50 and range from literature
Zinc	882.9 (754.8-1032.7) **50.4**	127.6 (67.8-240.1) **1.0**	1656.8 (1174.5-2337.2) **100**	169.7 (150.7-191.0) **3.8**	127.7 (107.4-151.9) **1.0**	13800 (n=1)
Copper	27.2 (22.0-33.7) **11.9**	113.3 (71.9-178.5) **100**	16.6 (14.9-18.5) **1.0**	19.6 (16.7-23.0) **4.1**	18.3 (15.7-21.3) **2.8**	7060 (n=3) (350-19500)
Cadmium	104.4 (83.7-130.3) **3.5**	78.6 (26.9-229.3) **1.0**	1115.6 (941.6-1321.8) **100**	132.0 (119.9-145.3) **6.2**	78.2 (67.7-90.4) **1.0**	22765 (n=2) (16030-29500)
Malathion	44.7 (30.8-64.9) **1.0**	11.8% inhibition at 40000 **100**	80840 (71550-91330) **100**	3.18 (2.36-4.27) **1.0**	1.14 (1.04-1.25) **1.0**	59700 (n=1)
2,4-D	>422000	252988 (127350-502573)	>422000	>422000	>422000	58433 (n=3) (13000-100700)
Endothall	18260 (13360-24960) **1.0**	>270000 **100**	>270000 **100**	66730 (55830-79760) **19.3**	48280 (40230-57930) **11.9**	--

other microbial test, Microtox® (Table 1). The use of a microplate reader with this test allows for the determination of definitive values for reading the chromogenic changes upon exposure to toxicants. Although the MetPLATE™ test was the only test that we performed in which we obtained an EC50 for 2,4-D, values obtained for toxicity of organics were usually much higher than those from the standard test. This test has been recommended for use with metal toxicity and is generally insensitive to organic toxicants (Bitton *et al.* 1994).

The ability to store cysts for long periods without the need to culture organisms makes the rotifer test attractive. This test, however, was relatively insensitive to the chemicals tested and would not be of much use for detecting toxicity at levels typically found in the environment. Ingestion rates by rotifers of fluorescent microspheres is a more rapid and sometimes more sensitive test than the 24-hour mortality test (Juchelka and Snell 1994) and may be a more useful test in some situations.

EC50's for Microtox® obtained from the literature (Awong *et al.* 1989, Dutka *et al.* 1983, Giesy *et al.* 1990, Gosh *et al.* 1997, McFeters *et al.* 1983, Somasundaran *et al.* 1990) are presented in Table 1. Overall, this test was among the least sensitive, although it may be more sensitive to organics than metals (Walker 1988). Sensitivity may be increased by using periods longer than five minutes for metal toxicity. Sensitivity for organic compounds, however, may decrease in some cases with increased exposure time (Gosh *et al.* 1997). This "recovery" effect has been noted with other organic compounds (Dutka *et al.* 1983, Walker 1988) and it has been suggested that this results from an almost instantaneous toxicity that is not progressive. Dutka *et al.* (1983) have suggested that the proportion of organisms emitting light remains constant but can show up as a slight recovery when the data are plotted.

We compared standard *C. dubia* tests with the IQ Toxicity Test™, MetPLATE™, and the rotifer test (Figure 1). We did not contrast Microtox® with the other tests because we lacked some comparable information. Results from testing with the herbicide 2,4-D were omitted from this analysis because little toxicity was detected with the toxicity test methods that *we* used. The 24-hour *C. dubia* test had the most sensitive ranking followed closely by the IQ Toxicity Test™ (Figure 1). The IQ Toxicity Test™ was the most sensitive of the very rapid tests and, in most cases, was within an order of magnitude of the LC50 calculated from 48-hour standard tests (Table 1). The MetPLATE™ test kit did well when compared with standard metal toxicity results but poorly when tested with organics (Table 1). This test has been promulgated as useful for detecting metal toxicity while being relatively insensitive to organics (Bitton *et al.* 1994) and our results support this assertion. The rotifer test was largely insensitive to both organics and metals, except for copper to which it was very sensitive (Table 1).

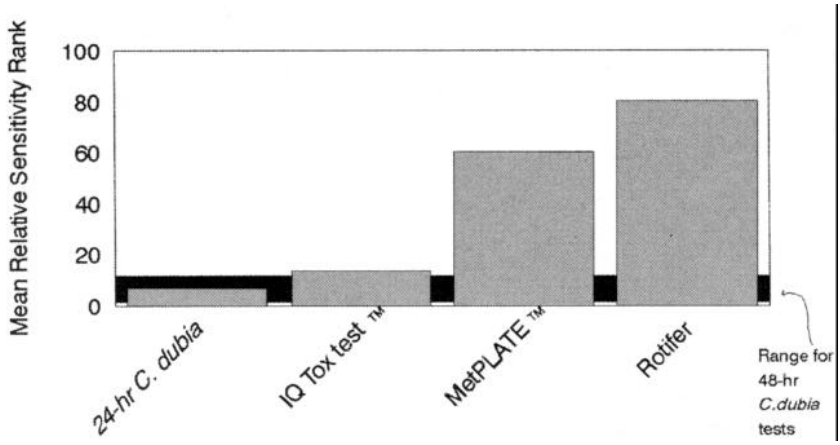

Figure 1. Mean (n=5) sensitivity rank of toxicity tests. Ranks determined from sensitivity (LC50/EC50 and greater than) values in Table 1.

Often toxicity testing during field surveys is done after a day of field work and is performed in a motel room, field office, or other remote site during the evening. Sometimes, travel the following day involves work at additional sites with the night spent in a new location. There are, of course, exceptions to this and some organizations maintain mobile bioassay laboratories that may have staff operating throughout the day with excellent ability and facilities to perform standard toxicity tests of 48-96 hours in duration. For the former example, however, even a 24-hour test may be too long, and it is unlikely that a 24-hour *C. dubia* or rotifer test could be accomplished. If this criterion is used, then only the IQ Toxicity Test™, MetPLATE™, and Microtox® tests could be used. Any one of these tests may be useful in a particular situation and no one test, despite our relative ranking of sensitivity (Figure l), will be sensitive to all compounds. Others have recommended using a suite of toxicity tests containing tests sensitive to different toxicants for detection of all possible toxicants. This may be less important when used adjunctly as part of a biosurvey because other measurements of impact (e.g., macroinvertebrate, fish, periphyton communities and habitat) already provide a "suite" of responses. In this situation a toxicity test could be selected that would show sensitivity to the expected problem (e.g., mining or smelting activity, IQ Toxicity Test™ or MetPLATE™; agricultural drains, IQ Toxicity Test™ or Microtox®).

Usually, the time to results was the only overall time savings for these rapid tests.

Although not a part of this study, the set up of a rapid toxicity test took as much time, or longer, than setup for a standard test. If time to results is not a constraint (as it is in field work) it may be more efficient to perform a standard toxicity test.

It should also be pointed out that sensitivity to pure chemicals in the laboratory may not directly translate to sensitivity to complex waters containing toxicants in the field. Janssen *et al.* (1993) found a lack of correlation in results of testing field effluents for toxicity between an enzymatic inhibition test with *Daphnia magna* and a 48-hour standard test. They suggested that some of this lack of correlation was because of food sources found in the effluents that satiated the daphnids during the l-hour exposure period and resulted in reduced ingestion of the enzyme substrate. Field collected waters that contain large amounts of natural food sources may need to be filtered before using this type of test kit. Anomalous results of this sort may be less of a problem with MetPLATE™. Although not compared with a standard test, Bitton *et al.* (1994) found that, in most cases, MetPLATE detected toxicity in ten waste and process waters that contained metals. It is obvious from the examples of Janssen *et al.* (1993) and Bitton *et al.* (1994) that more research is needed in comparative testing of toxins found in field situations and these rapid test kits.

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