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Predictive Ability of the *Daphnia magna* IQ Toxicity Test™ for Ten Diverse Water Treatment Additives

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The United States Environmental Protection Agency (USEPA), using a water quality based approach, has required 3,700 major dischargers to conduct biomonitoring as a condition of permitting (Weber *personal communication*). The Clean Water Act of 1977 (PL 95-217 Section 101(a)(3)) states "...it is a National policy that the discharge of toxic pollutants in toxic amounts be prohibited", thus mandating not only the monitoring and reporting of biomonitoring data but also the reduction of toxicity for those dischargers that are not in compliance.

The physiology, ecology, husbandry, and utility as a toxicity indicator organism of *Daphnia magna* have been well documented (Weisz 1967; USEPA 1991; Nebeker *et al.* 1986). The USEPA, American Society for Testing and Materials, American Public Health Association, Organization of Economic Cooperation and Development as well as other organizations have published guidlines to perform bioassays with *Daphnia magna* as the test species.

Unfortunately, conventional toxicity test methodologies do not provide data in real time. A sample taken at a treatment plant on 1 day may be toxic, but the toxicity will not be known for at least several days. Because of this problem, several rapid-test methodologies, such as Microtox™, Rotoxkit™, and Artoxkit™, generate IC50, EC50 and LC50 values, respectively. However, these tests employ the use of surrogate test species whose sensitivity to toxins may need to be related back to the species of primary concern. A new rapid test (the IQ Toxicity Test™) has been developed (Hayes 1992) that uses the species of concern, rather than a surrogate. This test assesses toxicity by observing *in vivo* inhibition of an enzyme in a test organism using the fluorometric biomarker methylumbelliferyl galactoside (MUF). Animals are exposed to a toxicant for 1 hr and then a solution of the biomarker substrate is added directly to the exposure chamber. The animals feed directly on the biomarker substrate if sufficiently starved. Those organisms with functional enzyme systems will be able to cleave the sugar from the florescent marker. The florescent marker is then free to move about in the hemolymph of the organism and is readily observed using a common battery-powered black light. The premise of the test is that animals that have been adversely affected by toxicity in the 1-hr exposure will have impaired

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galactosidase enzyme systems and will eventually die in a full 48-hr exposure. MUF sugar substrates have been used to assess microbial activity (Obst 1985), sediment toxicity (Burton *et al.* 1989) and exoenzymatic reactions (Hoppe 1983).

The objective of this study was to test the comparability of the *Daphnia Magna* IQ Toxicity Test™ and the 48-hr conventional *D. magna* toxicity test with several common water treatment chemicals.

MATERIALS AND METHODS

Samples of ten water treatment additives were provided by Betz Laboratories, Trevose, Pennsylvania. These additives are trade formulations commonly used for industrial water treatment purposes, and are described as follows: 1, a biocide comprised of an aqueous solution of 13% N-alkyl-dimethyl benzyl ammonium chloride; 2, an antimicrobial agent (proprietory formulation); 3, an aqueous solution of 40% polyquaternary amines (MW 2 x 10^4); 4, an aqueous solution of 18% polyquaternary amines (MW 3 x 10^5); 5, an aqueous solution of 15% ethoxylated alcohols; 6, 100% cyclohexamine; 7, a conditioning agent comprised of 34% ethoxylated alkyl phenols; 8, a phosphatizing additive (18% phosphoric acid in water); 9, an aqueous solution of 50% polyacrylate polymers; and 10, an aqueous solution of low molecular weight acrylate copolymers. Stock solutions were made of each additive with distilled water. No carriers were utilized. Samples of these materials can be obtained by contacting Aqua Survey, 499 Point Breeze Rd, Flemington, NJ 08822.

The primary test objective was to compare results from 1-hr enzymatic inhibition and 48-hr conventional studies. Two 1-hr tests and one 48-hr definitive study were performed at Laboratory A on each of the ten additives. Laboratory B performed a single 1-hr test on each of the ten additives. One-hr and 48-hr tests were performed concurrently for all additives except the acrylate copolymer. In total, each additive was tested three times with the enzymatic inhibition method and once with the conventional 48-hr methodology.

Laboratory A used less than 48-hr-old *Daphnia magna* that had been cultured in moderately hard reconstituted water (80 mg/L $CaCO_3$ hardness) and fed exclusively a diet of *Ankistrodesmus falcatus*. Laboratory B used less than 48-hr old *Daphnia magna* that had been cultured in hard reconstituted water (150-200 mg/L $CaCO_3$ hardness) and fed a diet of *Selenastrum capricornutum* and YTC (Yeast and Trout Chow mixture, USEPA 1991). Organisms used in 1-hr tests were starved by placing them in culture water devoid of food for a period of at least 6 hr prior to initiation of testing. The starvation period is required to ensure that the organisms will all feed on the substrate.) Organisms used in the 48-hr definitive studies were maintained in a food rich environment until test initiation. The IQ Toxicity Test™ (Aqua Survey Inc, Flemington, NJ) was performed according to manufacturers instructions (Aqua Survey 1989). A series of five test concentrations and a control were pipetted into 15-mL flat-bottomed borisilicate glass test tubes to a volume of 10 mL in triplicate. Six starved

Daphnia magna were pipetted into each tube. Care was taken to minimize the amount of carryover water during organism distribution. Culture water was used for dilution water. Organisms were exposed for 1 hr at ambient room temperature (20°C) and lighting (50-100 ft-c) and then 0.25 mL of substrate solution was forcefully added to each tube above the water line. Fifteen min later, each tube was illuminated with a longwave ultraviolet light source (hand-held, battery-powered blacklight). Tests were scored by comparing fluorescent intensity of treated organisms to the controls. An organism not fluorescing as brightly as the control organisms was considered adversely affected.

The 48-hr definitive tests were performed according to USEPA (1991) methods. A series of five test concentrations and a control was established in 250-mL borisilicate glass beakers with a final volume of 200 mL. The test was performed with two replicates having ten organisms per replicate. Organisms were exposed for 48 hr at 20°C with a 16L:8D photoperiod. New (devoid of food) culture water (type dependant on test laboratory, see above) was used for dilution water. Organisms that were not moving or exhibiting no movement after gentle prodding were scored as being dead.

Data were analyzed for toxicity using standard methodologies. The LC50 and EC50 values and their confidence limits were calculated using a set of USEPA computer programs known as the TOXDAT Multimethod, which uses the binomial, moving-average angle, and probit methods as appropriate (Stephan 1977). The coefficient of variation was corrected for the bias caused by the small sample size (Sokal and Rohlf 1981). Linear regression was calculated using Lotus 1-2-3, version 2.2.

RESULTS AND DISCUSSION

Observed toxicity ranged from one-half to several thousand parts per million (Table 1). In all cases, the IQ Toxicity Test™ was able to predict toxicity within the same order of magnitude as the conventional methodology. The correlation between the two test types was extremely high ($r^2 = 0.9997$). The 1-hr test appears to be repeatable, albeit this conclusion is based on only two tests. Differences in the results between laboratories may be due to a number of factors including differences in culture technique, culture and dilution water quality, organism health, and subtle differences in technique. Reported intralaboratory variations for round robin studies of conventional 48-hr tests are between 21 and 166 percent depending on the chemical used and the number of laboratories (Rue *et al.* 1988; USEPA 1991). More research is required to determine the precise reason for the variation and its implications for this test; however, the variation does not exceed that reported for conventional methodology and therefore would be acceptable for routine screening tests. The *Daphnia magna* IQ Toxicity Test™ was simple to use and provided data comparable to conventional methodologies in much less time. The test is conducted in less than 2 hr and appears to provide quick, inexpensive, toxicity screening of a wide variety of complex organic formulations. Research is ongoing to determine the applicablity to whole

Table 1. Results of toxicity tests with ten water treatment additives.
a = laboratory A, b = laboratory B. Intralaboratory variation precedes interlaboratory variation. Concentrations in mg/L.

Compound	1-hr EC50 (95% Confidence Limits)	48-Hr LC50 (95% Confidence Limits)
Biocide with cationic surfactants	0.15 (0.11 - 0.20)a 0.20 (0.12 - 0.31)a 0.73 (0.40 - 1.20)b CV = 22.5%,96.2%	0.40 (0.35 - 0.44)
Antimicrobial agent	0.26 (0.21 - 0.31)a 0.21 (0.08 - 0.15)a 0.07 (0.04 - 0.10)b CV = 16.8%,95.3%	0.56 (0.46 - 0.65)
Polyquaternary amine (mw = $2x10^4$)	0.29 (0.11 - 2.90)a 0.22 (0.13 - 0.39)a 0.56 (0.28 - 1.02)b CV = 21.6%,58.2%	0.18 (0.15 - 0.21)
Polyquaternary amine (mw = $3x10^5$)	6.00 (3.4 - 14.6)a 4.30 (1.9 - 10.9)a not conducted CV = 26.2%, NA	1.30 (1.06 - 1.60)
Ethoxylated alcohols	43 (35 - 54)a 43 (37 - 50)a 31 (24 - 40)b CV = 0%,25.8%	32 (28 - 36)
Cyclohexamine	45 (40 - 51)a 27 (21 - 34)a 41 (34 - 48)b CV = 39.8%,10.3%	83 (70 - 97)
Conditioning agent	31 (17 - 46)a 27 (20 - 35)a 117 (48 - 35000)b CV = 11.0%, 95.9%	75 (65 - 87)
Phosphatizing additive	110 (9 - 178)a 109 (66 - 151)a 125 (84 - 174)b CV = 0.7%,3.5%	89 (51 - 182)
Polyacrylate polymer	213 (175 - 267)a 197 (168 - 233)a 178 (130 - 253)b CV = 6.2%,3.5%	175 (100 - 250)
Acrylate copolymer	3500 (2600 - 5200)a 4800 (3900 - 7200)a 5000 (3200 - 10900)b CV = 25.5%,5.1%	3600 (3300-3800)

effluents and real time interpretation of chemical and treatment plant functions.

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