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TOXICITY DETERMINATION OF EXPLOSIVE CONTAMINATED SOIL LEACHATES TO DAPHNIA MAGNA USING AN ADAPTED TOXICITY CHARACTERISTIC LEACHING PROCEDURE

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An adapted toxicity characteristic leaching procedure was used to determine toxicity of soils to Daphnia magna. Soil samples were collected from U.S. military installations where open burning/open detonation (OB/OD) operations have occurred. The samples were extracted with CO_2-saturated distilled, deionized water equal to four times the mass of the soil. The CO_2-saturated water was most suitable for daphnia assays because pH adjustments of resultant extracts were seldom necessary. The possibility of toxic effects from materials necessary to adjust the pH, under conventional TCLP, was eliminated. The samples were extracted in darkness for 48 hr at 30 rpm end-over-end. Extracts were filtered through 0.45 μm membrane filters, serially diluted, and used in 48 hr acute daphnia toxicity assays. Metal and organic analyses were completed on each sample. Control samples of the same soil type as those at the OB/OD sites were located using USDA/SCS Soil Survey maps and sampled upwind of the OB/OD sites. Extracts from the Control samples proved to be nontoxic to daphnia at 100%. Sample extracts taken from the OB/OD sites were toxic to daphnia, having 48 hr acute EC_{50}s ranging from 1.2-69.4% (vol/vol).

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PREFACE

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TOXICITY DETERMINATION OF EXPLOSIVE CONTAMINATED SOIL LEACHATES TO DAPHNIA MAGNA USING AN ADAPTED TOXICITY CHARACTERISTIC LEACHING PROCEDURE

1. INTRODUCTION

Many military installations across the United States dispose of munitions through open burning/open detonation (OB/OD) operations. These operations deposit ash and residues onto the surrounding soils. The U.S. Army Chemical Research, Development and Engineering Center (CRDEC)* was tasked with the job of determining the relative toxicities of the soils at the four U.S. Army sites listed in Table 1. Daphnia magna were chosen as the test species to determine soil toxicity for the following reasons:

- They are sensitive aquatic organisms that are routinely used in determining the toxicity of mixtures.
- Toxicity comparisons between sites and open literature can be easily made.
- The methods available (with adaptations) for extracting contaminants out of the soils lends itself to aquatic testing with minimal modifications.[1]

Table 1. Sampling Sites

Radford Army Ammunition Plant (RAAP), VA
Milan Army Ammunition Plant (MAAP), TN
Pueblo Army Depot (PAD), CO
Anniston Army Depot (AAD), AL

An Adapted Toxicity Characteristic Leaching Procedure (ATCLP) was used in extracting munition residues from the soils before being subjected to daphnia toxicity tests. This procedure was adapted for use with daphnia testing by replacing acetic acid [typically used in Toxicity Characteristic Leaching Procedure (TCLP)] with a saturated carbonic acid solution, eliminating extreme pH effects.

*Now known as the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC).

Three variations of soil from each OB/OD site were used:

- Contaminated soil - soils from the OB/OD sites.
- Fortified soils - contaminated soils spiked with known concentrations of explosives.
- Control soils - uncontaminated soil samples located using U.S. Department of Agriculture/Soil Conservation Service (USDA/SCS) Soil Survey maps.

The objective of this study was to determine the toxicity of soil sample leachate from the OB/OD sites and compare the toxicity between sites using Daphnia magna.

2. METHODS

This study was conducted under Good Laboratory Practices (GLP). All testing conformed to U.S. Environmental Protection Agency (EPA)[2,3] and American Society for Testing and Materials (ASTM)[4] guidelines.

2.1 Leachate Procedure.

The TCLP in these experiments was adapted for use with daphnia acute toxicity tests by substituting CO_2-saturated water for acetic acid. Carbonated water was most suitable for daphnia assays because pH adjustment of the resultant extracts was seldom necessary.

Soil samples were collected at the various sites via surface collecting techniques described by Checkai and co-workers.* Control soil samples were located using USDA/SCS Soil Survey maps. Each soil sample was subsampled and weighed into a tared 1-L EP Tox Jar [e.g., those provided precleaned by Scientific Specialty Services (Randallstown, MD). Such jars are washed in laboratory-grade biodegradable, nonphosphate detergent, rinsed three times with tap water, rinsed with 1:1 nitric acid, rinsed three times with ASTM Type 1 deionized water, rinsed with hexane, and oven-dried]. Saturated carbonic acid solution (extracting solution) was added to the soil at a ratio equal to

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*Checkai, R.T., Wentsel, R.S., Phillips, C.T., and Yon, R.L., Controlled Environmental Soil-Core Microcosm Unit (CESMU) for Investigating Fate, Transport, and Transformation of Chemicals in Site-Specific Soils, MS-1110, U.S Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, submitted for publication 25 May 1993, UNCLASSIFIED Report.

four times the mass of the soil to be extracted. If the required mass of soil plus extracting solution exceeded 800 mL, the soil sample was divided equally by weight so that the 800 mL/jar limit was not exceeded. The samples were agitated in the dark for 48 hr at 30 rpm (rotary end-over-end) at a mean laboratory temperature of 20 ± 2 °C. After agitation was completed, the soil was allowed to settle. The supernatant was decanted and filtered through a 0.45 μm membrane filter and placed in a pre-cleaned EP Tox Jar.

2.2 Munition Residue Analysis.

Following ATCLP, the extract was analyzed for the compounds (shown in Table 2) using High Performance Liquid Chromatography (HPLC) equipped with a LC-8 reverse phase (RP) column using a wavelength setting of 224 nm. The mobile phase solvent used consisted of 62.4% H_2O, 37.5% methanol, and 0.03% tetrahydrofuran.

Table 2. Munition Residues Analyzed for in Extract

Cyclotetramethylenetetranitramine (HMX)
1,3,5-Trinitrobenzene (TNB)
Cyclotrimethylenetrinitramine (RDX)
2,4,6-Trinitrotoluene (TNT)
2,4-Dinitrotoluene (2,4-DNT)
2,6-Dinitrotoluene (2,6-DNT)

2.3 Metal Analysis.

Cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) were analyzed in the leachate directly after the filtering (0.45 μm) process from the ATCLP. Samples were injected directly and analyzed for total dissolved metals using Atomic Absorption Spectrophotometry (AAS) (Perkin-Elmer Model 3030). Blanks and standards were run periodically to monitor background contamination and instrument drift. Results were plotted against a standard curve and subjected to regression analysis to determine the concentration of metals in solution.

For a more detailed description of the analytical methods, see Checkai and co-workers.*,†

2.4 Daphnia Toxicity Assay.

Daphnia magna were obtained from Dr. Freida Taub, University of Washington, College of Ocean and Fishery Sciences, Seattle, WA. Daphnia were reared in the laboratory as described by Goulden and co-workers.[5] Daphnia stock cultures were fed a mixture of vitamin enriched Ankistrodesmus falcatus, Selenastrum capricornutum, and Chlamydomonas reinhardi. Daphnia culture media was derived from well water passed through a limestone pH adjustment tank, a Zeata Sol iron removal system, carbon filtration, and finally, through a UV sterilization system.

Hardness, conductivity, and pH measurements were taken using 80-100% extract (depending on the amount of extract available). Leachates from several of the soil samples resulted in having high pH that was adjusted (7.2-8.2) using 0.5 M NaOH, as shown in Table 3. All the extracts were tested within 72 hr after the extraction.

Ten daphnia, >24 hr old, were placed into 250-mL glass beakers filled with 100 mL of diluted extract solution. Concentrations ranged from 100-0.6% volume to volume (dilution series were a factor of 0.5 apart). The test beakers were placed in a temperature controlled room (20 ± 2 °C) with a light/dark cycle of 16/8 hr with 65 ft candles of light. Two replicates were used in each test. Daphnia were gently touched with a pasteur pipet at 24 and 48 hr. If the daphnia could not swim actively for 15 s, immobilization was recorded. The EC_{50} (effective concentration at which 50% of the organisms are immobilized) values were computed using the Probit analysis, as prepared by Kessler.[6] The EC_{50} was also tabulated graphically using a least square analysis. The graphically determined EC_{50} was used in verifying all Probit analyses.

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*Checkai, R.T., Mojor, M.A., Nwanguma, R.O., Phillips, C.T., and Sadusky, M.S., Transportation and Fate of Nitroaromatic and Nitramine Explosives in Soils from Open Burning/Open Detonation Operations, Pueblo Army Depot, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, unpublished data, May 1992.

†Checkai, R.T., Mojor, M.A., Nwanguma, R.O., Wentsel, R.S., and Sadusky, M.S., Transportation and Fate of Nitroaromatic and Nitramine Explosives in Soils from Open Burning/Open Detonation Operations, Radford Army Ammunition Plant, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, unpublished data, May 1992.

Table 3. Water Quality/Toxicology Data

Leachate	Conductivity (uMHOS)	Hardness (ppM)	pH	48 hr EC50 (vol/vol %)
RAAP Fortified	900 @ 100 %	252 @ 100 %	6.9 @ 100 %	11.4
RAAP Contaminated	825 @ 100 %	268 @ 100 %	6.8 @ 100 %	9.3
RAAP Control	500 @ 80 %	232 @ 80 %	5.9 @ 100 % *	NE**
MAAP Fortified	1200 @ 80 %	568 @ 80 %	7.0 @ 80 %	4.7
MAAP Contaminated	1200 @ 80 %	552 @ 80 %	7.0 @ 80 %	4.3
MAAP Control	240 @ 80 %	236 @ 80 %	5.0 @ 100 % *	NE
AAD Fortified	270 @ 100 %	Not Done	5.2 @ 100 % *	1.9
AAD Contaminated	360 @ 100 %	40 @ 100 %	5.5 @ 100 % *	1.2
AAD Control	400 @ 100 %	40 @ 100 %	5.1 @ 100 % *	NE
PAD Fortified	3200 @ 100 %	1300 @ 100 %	7.7 @ 100 %	5.2
PAD Contaminated	2900 @ 100 %	1200 @ 100 %	7.0 @ 100 %	9.4
PAD Control	1300 @ 100 %	180 @ 100 %	8.2 @ 100 %	NE

* The pH had to be adjusted within a range of 7.2 - 8.2 using 0.5M NaOH.
** NE - No Effect.

3. RESULTS

3.1 RAAP.

Concentrations of Zn and Cu were found in the leachates from RAAP soils as shown in Table 4. In Table 5, these metals were at or above the reported 48-hr EC_{50} values for daphnia. The munition residues of 2,4-DNT and 2,6-DNT found (Table 5) in the leachate from fortified RAAP soil were much higher than the concentrations found in the leachate from contaminated RAAP soil. The TNB and TNT residue concentrations from the fortified and contaminated leachates were approximately the same. However, the concentration of TNT is well below the EC_{50} values for daphnia (Table 5). The EC_{50} values between the fortified and the contaminated soil leachates were 11.4 and 9.3%, respectively (Table 3). Even though the fortified leachate had higher concentrations of 2,4-DNT and 2,6-DNT, the toxicities between the fortified and contaminated leachates were closer than expected. Therefore, the major contributors of toxicity from leachate from the RAAP site are Cu, Zn, and TNB combined.

3.2 MAAP.

Concentrations of Cd, Cu, and Zn were found in leachates from MAAP soils (Table 4). Copper and Cd levels were at reported EC_{50} values for daphnia, while Zn concentrations were several orders of magnitude higher than daphnia EC_{50} values (Table 5). When the MAAP soil was fortified with munitions, the concentrations of the residues in the leachate resulted in being approximately 89-100% higher than the residues in leachate from the contaminated soil (Table 4). The leachate from contaminated MAAP soil had relatively low concentrations of HMX, RDX, and TNT, which are some of the compounds that are least toxic to daphnia. Fortifying the soils with munitions did not increase the leachates' toxicity to daphnia. The EC_{50} values were 4.3% for the leachate from contaminated soils and 5.8% for the leachate from the fortified soils (Table 3). The hardness and conductivity were very high, and the pH was neutral. Based on these results, Cd, Cu, and Zn appear to be the major contributors to the toxicity of the leachates from the MAAP site.

Table 4. Organic and Metal Concentrations in Leachates

	Organics (mg/L)					
Site	HMX	TNB	RDX	TNT	2,4-DNT	2,6-DNT
RAAP Fortified	0.23	20.9	< 0.12	4.2	18.7	7.2
RAAP Contaminated	< 0.14*	19.2	< 0.12*	4.1	0.2	< 0.36*
MAAP Fortified	225	< 0.14*	248	3.5	222	89.5
MAAP Contaminated	3.0	< 0.14*	27.2	3.8	< 0.17*	< 0.36*
PAD Fortified	< 0.14*	3.43	< 0.12*	111	164	75.8
PAD Contaminated	< 0.14*	< 0.14*	< 0.12*	< 0.09*	< 0.17*	< 0.36*
AAD Fortified	5.2	37.2	46.1	90.6	173	76.8
AAD Contaminated	< 0.14*	< 0.14*	< 0.12*	< 0.09*	< 0.17*	< 0.36*

* Analytical Detection Limits

	Metals (mg/L)				
Site	Cd	Cr	Cu	Pb	Zn
RAAP Fortified/ Contaminated	< 0.01*	< 0.05*	0.03	< 0.01*	0.5
MAAP Fortified/ Contaminated	0.02	< 0.05*	0.03	< 0.01*	7.7
PAD Fortified/ Contaminated	0.01	< 0.05*	< 0.02*	< 0.01*	< 0.01*
AAD Fortified/ Contaminated	0.03	< 0.05*	0.25	< 0.01*	0.75

* Analytical Detection Limits

Table 5. Open Literature EC_{50} Values for Individual Compounds

Compound	Daphnia 48-Hr EC_{50} (mg/L)	Ref.
RDX	>100	7
HMX	>32	7
TNT	11.9	8
TNB	2.7	9
DNB	46	10
2,4-DNT	35	9
2,6-DNT	21.7	9
Cd	.030	11
Cr	.022	12
Cu	.031	13
Pb	4.4	14
Zn	.068	14

3.3 PAD.

The only metal detected in the leachate from PAD soil was Cd (0.01 mg/L), which was below the reported EC_{50} values for daphnia (0.03 mg/L). The hardness and conductivity were extremely high with a slightly basic pH (Table 3). The control soil leachate at 100% did not have an apparent effect on daphnia. Therefore, the elevated hardness and conductivity did not cause acclimation problems for daphnia. Munition residues (TNB, TNT, 2,4-DNT, 2,6-DNT) were detected in the leachate from fortified PAD soil (Table 4), and no residues were detected in the leachate from contaminated PAD soil. Leachate from contaminated PAD soil was the least toxic of all soils. Because only Cd (Table 5) was detected and munition residues were only found in the leachate from fortified PAD soil, it is assumed that the munition residues were the major contributors to the toxicity of the leachate from the fortified PAD soil. The leachate from the contaminated PAD soil was relatively nontoxic to daphnia.

3.4 AAD.

High concentrations of Cd, Cu, and Zn were found in AAD leachates. The Cu and Zn were well above published 48-hr EC_{50} values (Table 5). Munition residues found in the leachates were similar to that found in the PAD leachates. The fortified soil

leachate had high concentrations of munition residues, while munition residues in the leachates from the contaminated soil were below detectable limits (Table 4). Adding munitions tc the soil changed the toxicity of the leachate only slightly. The EC_{50} values for the fortified and contaminated leachate were 1.9 and 1.2%, respectively. The pH and hardness were low (Table 3), which tends to make metals more soluble, making the leachate much more toxic to the test organisms. Since the toxicity between the leachates from the contaminated and fortified soils from the AAD were approximately the same, the metal concentrations were the same, and no munition residues were detected in the contaminated leachate; the main contributors to the toxicity were the metals in solution with some influence from the residues.

All Control soil leachate samples were nontoxic to daphnia at 100% extract. The pH was adjusted on all the control leachate except the PAD sample. The hardness of the control leachates ranged from 40-236 ppm, which did not cause acclimation problems with the daphnia.

4. DISCUSSION

The daphnia is a small (1.0 to 4.0 mm) freshwater invertebrate that is commonly found in ponds, lakes and reservoirs. Daphnia magna is the largest species of daphnia and is found in the northern temperate zones of Canada and along portions of the west coast of the United States.[15] They filter-feed on algae. However, they do not discriminate between food type, only size. In the laboratory, the daphnia are maintained in a parthenogenetic reproductive mode,[16] reducing the variability within the population. Daphnia have been the species of choice for many types of assays across the country, not only due to the ease with which they are cultured, but more importantly, due to their sensitivity to environmental change and to toxic insult.

Daphnia are very sensitive to changes in water hardness, conductivity, and pH.[17] The standard practice when introducing daphnia to new media is to change over to the new media in four 25% increments lasting 24 hr each. The daphnia used in this study were cultured in water having a pH of 7.9, a conductivity of 400 µmhos, and a hardness of 98 ppm. The Control leachates from all the sites had hardnesses ranging from 40-236 ppm with conductivity ranges from 240-1300 µmhos. These wide ranges in water chemistry did not have an apparent effect on the daphnia at 100% leachate up to 48 hr. The leachates from the contaminated and fortified soils had hardnesses ranging from 40-1300 ppm with conductivity ranging from 240-3200 µmhos. Such extreme changes in water chemistry may create acclimation problems for daphnia, which may influence toxicity results. The direct effects of

pH were eliminated by adjusting the leachate (RAAP, MAAP, and AAD Controls, as shown in Table 3) to within the range of 7.2-8.2 before dilutions were made.

Metals are extremely toxic to aquatic organisms, as shown in Table 5, and are influenced by pH, hardness, conductivity, humic matter, and suspended sediments. Low pH and/or low hardness will increase metal toxicity to aquatic organisms.[7] However, if the hardness of the water is high and the pH is near neutral, the toxicity from metals is reduced. In part, this is due to the competition between the trace metal [Calcium (Ca)] and the hardness metal [Magnesium (Mg)] for the active sites on the cell membrane.[9] Excess trace metals on the membranes alter the effectiveness of gas exchange, and the organisms die from respiratory complications. However, in natural waters, trace metals typically form stable hydroxy or carbonate complexes, and only a small fraction of the total concentration remains in an available form.[18] Humic materials and suspended clays reduce the effects of metals on aquatic organisms. These materials complex the metals, reducing their ability to bind to active sites in the cell membrane. In most cases, trace metals are deposited into bottom sediments, rendering them relatively harmless to pelagic organisms. However, bottom and subsurface dwellers may be subjected to toxic insult through ingestion and dermal contact.

The concentration of TNB residues found in the leachates from contaminated and fortified RAAP soil, were higher than the reported EC_{50} values. All other explosive residues were below EC_{50} values. The elevated concentrations of munition residues in the leachate from the fortified RAAP soil did not noticeably increase the EC_{50}. Therefore, it is assumed that the combination of Cu, Zn, and TNB in the leachate of both the fortified and contaminated RAAP soils are the major contributors to the toxicity.

The leachate from the MAAP soils had concentrations of Zn approximately 110 times more than published EC_{50} values (Table 5). Because the hardness was high and the pH was neutral, toxic effects of trace metals would be somewhat lessened. The leachate from MAAP fortified soils had a very high concentration (3.5-248 ppm) of munition residues, which resulted in this leachate being approximately twice as toxic as the leachate from contaminated MAAP soil.

Leachates from the PAD soils had the highest pH, hardness, and conductivity. However, there was only one metal detected (Cd), and the concentration was below published EC_{50} values. The combination of high pH and hardness and low metal concentration would result in the metal not having much influence on the toxicity of the leachate. Concentrations of munition residues were detected at 3.4-164 ppm in the leachate from fortified soil and below detectable limits in leachate from the

contaminated soil. Since the leachate from the PAD contaminated soil is 92% less toxic than the leachate from the fortified soil, it is assumed that munition residues are the major contributors to toxicity.

Leachate from the AAD soil had the lowest pH, hardness, and conductivity. Therefore, trace metals in solution would have an elevated toxic effect on daphnia. The metal concentrations detected in AAD leachate were above reported EC_{50} values, and munition residues were only detected in the leachate from the fortified soil. Yet, the EC_{50} values for the leachates from both the fortified and contaminated AAD soils were approximately the same. Thus, trace metals in the AAD leachates are suspected to be the major contributor to the toxicity.

Below is a ranked order of leachates starting with the least toxic:

Control leachate < PAD contaminated < RAAP fortified < RAAP contaminated < MAAP fortified < PAD fortified < MAAP contaminated < AAD fortified < AAD contaminated.

The explosives used to spike the soils are much less toxic to daphnia than the metals with the exception of Lead (Pb) (Table 5). In many cases, the concentrations of explosive residues detected in the leachates were much higher than the published EC_{50} values, which would suggest a major source of toxicity. However, in the case of the leachate from the AAD soils, fortifying the soil did not change the toxicity. This indicates that the toxicities of the metals and explosive residues in soil leachates are not additive.

The chemistry of mixture toxicology has very complex interactions. Comparing the toxicity of individual compounds (Table 5) to mixtures may yield erroneous conclusions. The simplifying assumptions made in this report allow relative comparisons of leachate toxicities. However, there may be undetected contaminants present in the leachates that may also be contributing to the leachates' toxicity.

5. CONCLUSIONS

As a result of the study conducted, the following conclusions are provided.

- All control soil leachates were nontoxic to daphnia.
- The Pueblo Army Depot (PAD) contaminated leachate was the least toxic.

- The Anniston Army Depot (AAD) fortified and contaminated leachates were the most toxic.

- Other contaminants not looked for may also be contributing to the toxicity of the leachates.

- Metals were detected at varying concentrations in all the leachates.

LITERATURE CITED

1. Green, G.C., Bartels, C.L., Hicks, W.J., Parkhurst, B.R., Linder, G.L., and Peterson, S.A., Protocols for Short Term Toxicity Screening of Hazardous Waste Sites, EPA-600/3-88/029, U.S. Environmental Protection Agency, Washington, DC, February 1989.

2. Users Guide: Procedures for Conducting *Daphnia magna* Toxicity Bioassays, EPA-660/8-87/011, U.S. Environmental Protection Agency, Washington, DC, March 1987.

3. Methods for Acute Toxicity Tests With Fish, Macroinvertebrates and Amphibians, EPA-660/3-75/009, U.S. Environmental Protection Agency, Washington, DC, 1975.

4. Guide for Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates and Amphibians, ASTM Standard E729, American Society for Testing and Materials, Philadelphia, PA, 1986.

5. Goulden, C.E., Conotto, R.M., Hendrickson, J.A., Jr., Hornig, L.L., and Johnson, K.L., Procedures and Recommendations for the Culture and Use of *Daphnia magna* in Bioassay Studies, ASTM Special Technical Report Publication 766, pp 139-160, American Society for Testing and Materials, Philadelphia, PA, 1982.

6. Kessler, F., Probit Analysis, U.S. Environmental Protection Agency, Cincinnati, OH.

7.Adams, T., and Sanders, J.R., "The Effects of pH on the Release to Solutions of Zinc, Copper and Nickel From Metal-Loaded Sewage Sludges," Env. Poll. Ser. B, Vol. 8, pp 85-99 (1984).

8.Pagenkopf, G.K., "Gill Surface Interaction Model for Trace-Metal Toxicity to Fishes: Role of Complexation, pH, and Water Hardness," Env. Sci. and Tech. Vol 17. (6), pp 342-347 (1983).

9.Bentley, R.E., LeBlanc, G.A., Hollister, I.A., and Sleight, B.H., Acute Toxicity of 1,3,5,7-Tetranitrooctahydro-1,3,5,7-Tetrazocine (HMX) to Aquatic Organisms, U.S. Army Medical Research Development Command, Washington, DC, 1977, National Technical Information Service Index 78 (18), 81:23, AD-A054.

10.Bailey, H.C., and Liu, D.H.W., *Lumbriculus variegatus*, a Benthic Oligochaete, as a Bioassay Organism, ASTM STP 707, Eaton, Parrish, and Hendricks, Eds., pp 205-215, American Society for Testing and Materials, Philadelphia, PA, 1980.

11. Pearson, J.G., Glennon, J.P., Barkley, J.J., and Hightill, J.W., "An Approach to the Toxicological Evaluation of a Complex Industrial Wastewater," ASTM STP 667, Marking and Kimerle, Eds., Aqu. Tox. pp 284-301, American Society for Testing and Materials, Philadelphia, PA, 1979.

12. Hermens, J., Canton, H., Steyger, W., and Wegman, R., "Joint Effects of a Mixture of 14 Chemicals on Mortality and Inhibition of Reproduction of *Daphnia magna*," Aqu. Tox. Vol. 5 (4), pp 315-322 (1984).

13. Schuytema, G.S., Nelson, P.O., Malueg, K.W., Nebeker, A.V., Krawczyk, D.F., and Ratcliff, A.K., "Toxicity of Cadmium in Water and Sediment Slurries To Daphnia," Env. Tox. and Chem. Vol. 3 (2), pp 293-308 (1984).

14. Mount, D.I., and Norberg, T.J., "A Seven Day Life Cycle Cladocern Toxicity Test," Env. Tox. and Chem. Vol. 3 (3), pp 425-434 (1984).

15. Taub, F.B., A Critical Review of Daphnia Ecology and Culture, EPA CR807209-01-1, U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, Washington, DC, July 1982.

16. Pennak, W.R., Fresh-Water Invertebrates of the United States, 2nd ed., John Wiley & Sons, New York, NY, 1978.

17. Goulden, C.E., and Henry, L., Daphnia Bioassay Workshop, Academy of Natural Sciences, Division of Environmental Research, Philadelphia, PA, 1988.

18. Borgmann, U., and Ralph, K.M., "Complexation and Toxicity of Copper and the Free Metal Bioassay Technique," Water Res. Vol. 17 (11), pp 1697-1703 (1984).