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Toxicity Identification Evaluation Using a Short-Term Chronic Test with *Ceriodaphnia dubia*

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EPA's Technical Support Document for Water Quality-Based Toxics Control (EPA, 1991a) defines a Toxicity Identification Evaluation (TIE) as a step-wise process which combines toxicity testing and analysis of the physical and chemical characteristics of causative toxicants. Since a TIE is conducted in a tiered approach, judgement is required in selecting the appropriate fractionation procedures. The TIE programs utilizing acute tests as an indicator of toxicity proved to be successful in characterization of challenging constituents like organic ions or surfactants (Jop et al. 1991a; Ankley and Burkhard 1992). The short-term chronic method with *Ceriodaphnia dubia* is significantly more sensitive than any acute test (Spehar and Fiandt 1986; Kszos et al. 1992). Changes in the sample matrix due to fractionation procedures may affect the performance of daphnids, making interpretation of the test results difficult or even impossible. Introduction of artificial toxicity to *C. dubia* from ion exchange columns was presented by Jop et al. (1992). The chemical analyses of the eluate from the exchange column revealed chemicals initially not present in the effluent and changes in concentration of the major ions. The chemicals eluted from the column as well as a new ratio not always typical for freshwater can produce toxic effect to daphnids.

The overall objective of this study was to identify and characterize the toxic constituent(s) in a process water which proved chronically toxic to the daphnid *C. dubia*. In addition, the chronic toxicity of four major ions (magnesium, calcium, potassium and sodium) was estimated in eluate from ion exchange column.

MATERIALS AND METHODS

The wastewater used for this study was treated in a plant constructed in 1992 and designed to treat process and cooling waters. During the treatment process, the wastewaters are first screened to remove coarse materials. After removal of glass particles and sand, the wastewater enters the anaerobic digester. The biodegradation of organic compounds by anaerobic digestion requires nutrient addition, therefore, nitrogen as ammonium hydroxide and phosphorus as

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phosphoric acid are added to the anaerobic equalization tank. During this process methane gas and sludge are produced. The influent from the anaerobic digester is further treated in an aerobic sequencing batch reactor - Continuous Activated Sludge System (CASS). At this point of the treatment, two influents, the cooling and wash water join the process water. The influent from the CASS basin is pumped to the clarifier and is mixed with alum and polymer. Alum is added to remove phosphate ions while the polymer acts as a coagulant of the aluminum phosphate. Subsequently, the influent is sand filtered to remove excess solids. Finally, the effluent is aerated and disinfected by passage through UV light. The final effluent is discharged to the receiving stream.

The identification of toxicity was based on the principal of sequential removal of a chemical fraction coupled with short-term, static-renewal toxicity tests with *C. dubia* of the fractionated wastewater. The TIE procedures were conducted according to Jop et al. (1991b). Fractionation treatments used for identification of toxic compounds include filtration, XAD-resin (Sigma), nitrogen purging, chelation with EDTA and TMT - trimercaptotriazine (Degussa), cation and anion resins, activated carbon, C-18 column, sublation, dichloromethane extraction and clinoptilolite (Steelhead) treatments. Corresponding blanks of each fraction were also tested for toxicity to ensure that sample manipulation did not introduce artificial toxicity. Preliminary results from short-term toxicity tests with *C. dubia* indicated that daphnids survival and reproduction was affected only in undiluted effluent, therefore, a standard short-term chronic test (EPA 1989) was used for testing.

During the confirmation procedures, the eluate from a clinoptilolite column was spiked with increasing concentrations of calcium, magnesium, potassium and sodium. The toxicity tests were conducted with the individual eluate samples fortified with 50, 100, 150, 200 and 300 mg/L potassium, 320, 420, 520, and 620 mg/L of sodium, 100, 200, 400, and 600 mg/L of magnesium and 100, 200 and 400 mg/L of calcium. The chronic toxicity was evaluated in a total of sixteen samples. Because a total hardness of the first nine samples (sample numbers 1,2,3,4,5,6,7,8,9) ranged between 12 and 28 mg/L as $CaCO_3$, a soft reconstituted water (hardness 58 to 60 mg/L, alkalinity 40 to 43 mg/L as $CaCO_3$) was used in three control treatments, while for the remaining seven samples (sample numbers 10,11,12,13,14,15,16) a hard reconstituted water (hardness 174 mg/L and alkalinity 123 mg/L as $CaCO_3$) was used as a control treatment.

Ceriodaphnia dubia ($\leq$ 24 hr old) were used as the test organisms. Daphnids were cultured in aged, soft and hard well water with hardness raised by the addition of reagent grade chemicals according to the EPA method for soft or hard water (EPA 1991b). *C. dubia* cultures were fed suspensions of a mixture of unicellular green algae (*Selenastrum capricornutum*) and YCT (yeast, Trout Chow and Cerophyll) once a day. Twenty-four hours before test initiation, all immature daphnids were removed from the culture beakers. Offspring produced over the first 8-hr period were culled individually using a glass pipet.

The short-term test with *C. dubia* was conducted in plastic cups (30 mL), each containing 15 mL of test solution and one daphnid in a water bath. Test conditions included a temperature of 25 $\pm$ 1°C and 16:8-h light and dark

photoperiod. The test included up to five effluent concentrations plus a dilution water control. Each treatment consisted of ten replicates each containing one daphnid. The renewal of the test solution was conducted daily by transferring the adult organisms to the freshly prepared solutions. During the renewal process, each cup was examined and the number of offspring produced over a 24-hour period was recorded. The *C. dubia* were fed 100 μL of algae *S. capricornutum*, and 100 μL of YCT suspension.

RESULTS AND DISCUSSION

Chemical analyses performed prior to initiation of the TIE program revealed no detectable (detection limit was 2-5 μg/L) concentration of copper, lead, nickel, silver, arsenic, zinc, selenium and 2.4 mg/L of magnesium, 13.0 mg/L calcium, 37.8 mg/L of sodium and 286 mg/L of potassium. The results of the short term chronic test with the first sample indicated that survival of *C. dubia* was affected in the whole sample (100%), while reproduction of daphnids expressed as number of offsprings produced per females was not significantly lower in any test concentration with the exception of 100%, compared to the reproduction of the control organisms. Toxicity of a pressure-filtered, XAD, EDTA, TMT, C-18 eluate, nitrogen purge, activated carbon, dichloromethane extraction, anion and cation were similar and none of the treatments applied to this sample removed toxicity. Daphnids survival and/or reproduction were affected in all applied treatments (Table 1). Survival and/or reproduction of daphnids exposed to the blank samples from C-18, anion and cation columns were affected, indicating introduction of artificial toxicity. Consequently, the eluate samples were screened for presence of organics using GC and HPLC. The analyses confirmed presence of several compounds most likely polar organics which were not present in original samples.

The second sample of process water was less toxic than the first sample. Survival of daphnids was not affected in any concentration while reproduction was reduced in the whole (100%) compared to controls. The concentration of calcium, magnesium, sodium and potassium in that sample was 18 mg/L, 2 mg/L, 14 mg/L and 220 mg/L, respectively. This time fractionation included ion exchange treatment with the clinoptilolite column. The hardness of the eluate from the clinoptilolite column was adjusted to 80 mg/L, 144 mg/L, and 180 mg/L. Survival of *C. dubia* in these three eluate samples ranged from 90% to 100%, while reproduction ranged from 16 to 21 offspring per female and was not significantly different from the control. Clinoptilolite resin removed the source of the chronic toxicity from process water. The chemical analysis performed on the eluate from clinoptilolite column revealed that potassium was exchanged for sodium. After passing process water through resing the concentration of calcium was 4.1 mg/L, potassium 8.4 mg/L, magnesium 1.8 mg/L and sodium 220 mg/L (Table 2). Potassium was identified as a likely source of the chronic toxicity in process water.

Ions like potassium, sodium, calcium or magnesium, which are physiologically essential for freshwater species, in large quantities may cause toxicity to these organisms. A freshwater invertebrate like daphnia may lack the osmoregulatory capacity to maintain cellular ionic conditions in the presence of increased concentration or absence of divalent or monovalent cations. Although, potassium most likely was responsible for the observed toxicity, still a confirmation procedure

Table 1. Summary of mean survival (%) and reproduction (number of offspring/female) at termination of the short-term static renewal toxicity tests exposing *Ceriodaphnia dubia* to individual fractions of the process water.

Fraction	Mean Survival (%)	Mean Number of Offsprings per Female
Whole		
Control (first)	100	21
50%	100	22
100%	0	0
Filtered	10	0
Blank	100	32
XAD	0	0
Blank	100	27
N_2 Purge	0	0
Blank	86	26
TMT	0	0
Blank	100	28
C-18 eluate	0	0
Blank	70	3
Anion	0	0
Blank	90	12
Cation	0	0
Blank	50	9
Na_2EDTA	40	3
Blank	100	23
Sublation	100	15
Activated carbon	80	9
Blank	90	23
Dichloromethane extraction		
pH 3 - aqueous	80	7
pH 7 - aqueous	50	4
pH 11 - aqueous	30	3
Whole		
Control (second)	90	20
50%	100	24
100%	70	6
Clinoptilolite		
Hardness 80 mg/L	100	18
Hardness 144 mg/L	90	16
Hardness 180 mg/L	100	21
Blank	100	14

which included fortification of the eluate from the clinoptilolite column with four major ions potassium, sodium, magnesium and calcium was warranted.

Clinoptilolite is a natural mineral comprised of sodium-calcium-aluminium-silicate arranged in an interconnecting lattice structure which gives it a unique ability to reversibly adsorb/desorb cations based on ion selectivity. The affinity of clinoptilolite to selectively take up ammonium ions makes it highly effective in controlling ammonia concentration in water; however, as an exchange resin,

Table 2. Concentration of sodium, potassium, magnesium and calcium in sixteen eluate samples from the clinoptilolite column. *All values expressed as mg/L.*

Sample ID	Sodium		Potassium		Magnesium		Calcium	
	Nominal	Measured	Nominal	Measured	Nominal	Measured	Nominal	Measured
Clinoptilolite eluate		220		8.4		1.8		4.1
1	220	227	58	58	2	1.6	4	3.8
2	220	230	108	116	2	1.5	4	4
3	220	239	158	162	2	1	4	3.9
4	220	234	208	217	2	1.6	4	3.7
5	220	232	308	326	2	0.6	4	4
6	320	323	8	5.9	2	0.8	4	3.9
7	420	408	8	6.6	2	1.5	4	4
8	520	490	8	6.6	2	1.4	4	4
9	620	620	8	7.2	2	1.3	4	3.9
10	220	240	8	9.0	102	103	4	4.4
11	220	240	8	7.8	202	208	4	4.2
12	220	242	8	6.6	402	396	4	4.5
13	220	234	8	11.4	602	620	4	4.9
14	220	230	8	7.8	2	3.2	104	103
15	220	230	8	6.6	2	1.8	204	206
16	220	231	8	6.6	2	1.2	404	410

clinoptilolite effectively removes some cations adding back other cations to the sample. The efficiency of a cation removal is dependent primarily upon its concentration in the effluent, pH, temperature, contact time, concentration of other competing ions and molecular size. The following cations (according to selectivity for various cations) can be removed using clinoptilolite resin: Rb, Li, K, Cs, NH_4, Na, Ag, Cd, Pb, Zn, Ba, Sr, Cu, Ca, Hg, Mg, Fe, Co, Al Cr. Besides removal of the cations, exchange columns like clinoptilolite have the potential to adsorb organics in capillaries of the mineral bead. Therefore, changes in toxicity in the clinoptilolite eluate samples are most likely attributed to changes in potassium concentration but they can be attributed to removal of other elements besides potassium from the sample matrix.

Increased concentrations of the monovalent ions, potassium and sodium in the eluate from clinoptilolite column combined with limited magnesium and calcium concentrations affected daphnids survival and reproduction. Survival of *C. dubia* was affected at 162 mg K/L and at 323 mg Na/L, while reproduction was reduced in 116 mg K/L and 220 mg Na/L. (Figure 1). Increased concentration of magnesium affected daphnid survival at 103 mg/L, while reproduction was affected

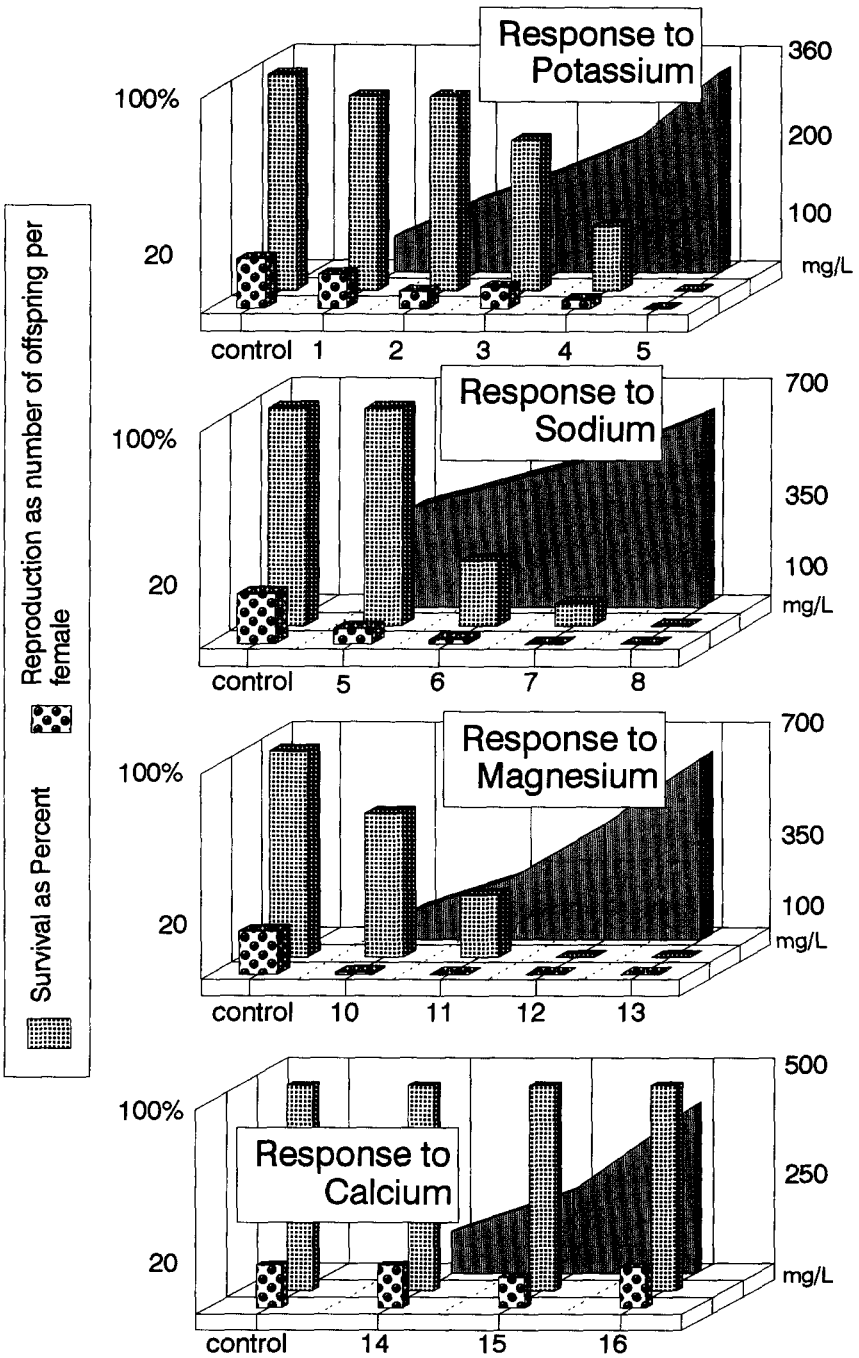

Figure 1. Biological response (survival and reproduction) of *Cerodaphnia dubia* to increasing concentrations of potassium, sodium, magnesium and calcium in eluate samples from the clinoptilolite column.

below 103 mg/L. Addition of calcium did not produce a biological affect up to 404 mg/L.

The ratio of monovalent ions to divalent ions is very important for maintaining balance and buffer capacity in water. It seems that the ratio between monovalent and divalent ions as well as the ratio between sodium and potassium or calcium and magnesium plays an important role in daphnids physiological processes. The safe concentration of sodium ranged from 1 to 150 mg/L, potassium from 1 to 100 mg/L, magnesium from 1 to 50 mg/L and calcium from 1 to 400 mg/L. At the same time the preferable physiological concentration of these ions for *C. dubia* ranged from 5 to 100 mg/L for sodium, 1 to 50 mg/L for potassium, 1 to 25 mg/L for magnesium and 50 to 300 mg/L for calcium.

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REFERENCES

Ankley GT, Burkhard LP (1992) Identification of surfactants as toxicants in a primary effluent. Environ Toxicol Chem 11:1235-1248

Jop KM, Kendall TZ, Askew AM, Foster RB (1991a) Use of fractionation procedures and extensive chemical analysis for toxicity identification of a chemical plant effluent. Environ Toxicol Chem 10:981-990

Jop KM, Foster RB, Askew AM (1991b) Factors affecting toxicity identification evaluation: the role of source water used in industrial processes. Aquatic Toxicology and Risk Assessment: Fourteenth Symposium ASTM STP 1124. In: Mayes MA and MG Barron, eds. American Society for Testing and Materials, Philadelphia, pp 84-93

Jop KM, Askew AM, Terrio KF, Simoes AT (1992) Application of the short-term chronic test with *Ceriodaphnia dubia* in identifying sources of toxicity in industrial wastewaters. Bull Environ Contam Toxicol 49:765-771

Kszos LA, Stewart AJ, Taylor PA (1992) An evaluation of nickel toxicity to *Ceriodaphnia dubia* and *Daphnia magna* in a contaminated stream and laboratory tests. Environ Toxicol Chem 11:1001-1012

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

U.S. Environmental Protection Agency (1989) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring Systems Laboratory, Cincinnati, OH

U.S. Environmental Protection Agency (1991a) Technical support document for water quality-based toxics control. Office of Water, Washington, DC.

U.S. Environmental Protection Agency (1991b) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Office of Research and Development, Washington, DC