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Acute Toxicity of Cadmium and Sodium Pentachlorophenate to Daphnids and Fish

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When estimating the toxicity of effluents it is desirable to use organisms sensitive to a wide range of pollutants. Currently, the U.S. Environmental Protection Agency (U.S. EPA) recommends the use of Daphnia pulex, Daphnia magna, and Pimephales promelas to assess the toxicity of freshwater effluents. Ceriodaphnia sp. has also received increased attention as a standard toxicity test organism due to its sensitivity, short generation time, and ubiquitous distribution.

Comparison of toxicity data generated by different investigators is often difficult because of differences in test procedures, dilution waters, or nutritional history of test organisms. To validly compare toxicity data from different sources, the sensitivity and variability of test organism response must be determined. Reference toxicants are well-defined, toxic substances used to compare the response of organisms to a toxicant through time and/or at different locations. Reference toxicants can also be used to estimate the condition of organisms at the time of testing (Marking 1966; Davis and Hoos 1975). Toxicity tests using prescribed methods and reference toxicants are slowly becoming routine practice to address differences and changes in species sensitivity.

The primary objectives of this research were to compare the sensitivity of Daphnia magna, Daphnia pulex, Ceriodaphnia reticulata, and Pimephales promelas to the reference toxicants $CdCl_2$ and sodium pentachlorophenate (NaPCP) and to compare results with those obtained by other investigators. A secondary objective of this study was to evaluate the effect of different dilution waters on the acute toxicity of these reference toxicants to the above test organisms.

MATERIALS AND METHODS

Daphnia magna and Ceriodaphnia reticulata were obtained from the U.S. Fish and Wildlife Service's Columbia National Fisheries Research Laboratory in Columbia, MO. D. pulex were obtained

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from the U.S. EPA Laboratory in Wheeling, WV. These organisms were cultured in 10-L glass aquaria containing reconstituted water or water from a deep well at the Johns Hopkins University, Applied Physics Laboratories facility in Shady Side, MD. Selected water quality characteristics of culture waters are presented in Table 1. Cultures were maintained under 390-540 lm/m^2 and an L:D 12h:12h photoperiod in walk-in environmental chambers held at 21 ± 1°C. All organisms received 1.5 mL of "Daphnia Chow" per liter of culture water four times a week. Additionally, *C. reticulata* received 2.5 x 10^4 *Selenastrum capricornutum* cells/mL culture media once a week while *D. pulex* and *D. magna* received 2.5 x 10^4 *S. capricornutum* cells/mL culture media three times weekly. All cultures were continuously aerated and ≈50% of the culture water was replaced weekly.

Pimephales promelas were obtained from U.S. EPA, Newtown, OH. Adult fathead minnows were maintained in well water at 20 ± 2°C under a photoperiod of L:D 16h:8h and fed twice daily with a mixture (≈50%) of trout chow (Ziegler Bros., Gardners, PA) and Tetra Standard Mix.

Table 1. Selected characteristics of waters used to culture test organisms and as diluents in toxicity tests.

Parameter	Water Type Reconstituted	Well Water*
pH at 22°C	7.8 ± 0.3	7.7 ± 0.4
Total Hardness (mg/L $CaCO_3$)	120 ± 10	200 ± 10
Total Alkalinity (mg/L $CaCO_3$)	110 ± 10	140 ± 10
Conductivity (μmhos/cm^2)	560	340

* Previous analyses showed negligible levels of heavy metals.

Spawning was carried out under the above conditions to obtain larval fatheads for use in toxicity tests. Larvae were fed *Artemia* sp. (<24 h) and larval fish food. This diet was supplemented with finely-ground Tetra Standard Mix and live *C. reticulata* when *P. promelas* reached one week of age.

Acute static toxicity tests with $CdCl_2$ and NaPCP were conducted according to U.S. EPA procedures. These reference toxicants were obtained from the U.S. EPA, Quality Assurance Branch, Cincinnati, OH. All four species were tested in 400-mL glass beakers containing 300 mL of test solution. Five toxicant exposures and a control were used in all experiments. Concentrations of Cd and NaPCP were nominal concentrations obtained by diluting the reference toxicants in deep well water or reconstituted water Diluents were the same waters used to culture test organisms. *P. promelas* tested in reconstituted water were acclimated to the diluent for one week prior to testing. Selected water quality parameters were monitored according to *Standard Methods*.

D. magna and D. pulex were <24 h old, C. reticulata <6 h old, and P. promelas 14 to 30 d old at the start of all experiments. In most cases, 10 invertebrates per exposure were used without replication. Twenty P. promelas (2 replicates of 10), were used per exposure during the larval fish tests. Lack of detectable movement was used as the criterion for mortality in all experiments. Test duration was 48 h for invertebrates and 96 h for P. promelas. Dead P. promelas were removed from beakers at 24-h intervals. Effects were determined at 24-h intervals in all experiments.

Concentration-mortality data were analyzed by a computer program calculating LC50 and 95% confidence intervals (CI) using the binomial and probit procedures. The criterion of nonoverlapping 95% CIs was used to determine statistical differences in LC50 values.

RESULTS AND DISCUSSION

Water quality parameters monitored during toxicity tests revealed that pH, hardness, and alkalinity were within the range of values reported in Table 1. The maximum change in pH during any one test was an increase of 0.6 units. The pH remained at ± 0.2 units of the initial value in remaining tests.

Results of Cd toxicity tests are presented in Table 2. Control mortality was ≤ 10% in all tests. As determined by the binomial method, no differences in species sensitivity to Cd are apparent for tests using the same water type and the different water types did not affect Cd toxicity to any given species. Results of probit analyses indicate that D. magna in the first experiment with reconstituted water were significantly more sensitive to Cd than D. magna in the second experiment with well water. However, this difference can not be attributed to differences in water types (i.e., higher well water hardness) because a significant difference does not exist between the LC50 of the second D. magna/reconstituted water experiment and D. magna experiments in well water. Additionally, a significant difference exists between replicate experiments with D. magna in well water, indicating some degree of variability in these experiments. As determined by probit analyses, the LC50 for D. magna in the first well water experiment was significantly lower than the D. pulex and P. promelas LC50s. However, when results of the second D. magna/well water experiment are considered, significant differences between species do not occur. When viewing these data collectively, it can be seen that the four species tested had similar sensitivities to Cd and that water type did not influence the results of Cd toxicity tests. These data allso illustrate the need for replicate experiments when evaluating differences in species sensitivity.

Results of toxicity tests with NaPCP are presented in Table 3. Control mortality was ≤10% in all tests. Statistically significant differences do not exist between the sensitivity of each of the invertebrates to NaPCP. P. promelas tested in well water was significantly more sensitive to NaPCP when compared with the three invertebrates. The higher degree of sensitivity of P. promelas to

Table 2. Results of acute toxicity tests with Cd. The same letters on data points indicate statistically significant differences between different species tested in the same water type. The same numbers on data points indicate significant differences between the same species tested in different waters. LC50 data for invertebrates are for 48 h tests; LC50 data for *P. promelas* are 48- or 96-h tests as indicated.

Organism	Water Type	Exp. No.	LC50 (95% Confidence intervals) mg/L Binomial	Probit
C. reticulata	Recon	1	0.11 (0.05-0.40)	-
D. magna	Recon	1	0.02 (NC)	0.02 (0.004-0.03)[1]
D. magna	Recon	2	0.05 (0.01-0.15)	0.04 (0.02-0.07)
D. pulex	Recon	1	0.08 (0.05-0.15)	-
D. pulex	Recon	2	0.10 (0.05-0.15)	0.07 (0-infinity)
P. promelas	Recon[x]	1	>0.15 (NC)	>0.15 (NC)
P. promelas	Recon[y]	1	>0.15 (NC)	>0.15 (NC)
C. reticulata	Well	1	0.09 (0.05-0.15)	-
C. reticulata	Well	2	0.07 (0.05-0.10)	-
D. magna	Well	1	0.05 (NC)	0.03 (0.01-0.05)[a,b,c,d]
D. magna	Well	2	0.08 (0.05-0.15)	0.08 (0.06-0.10)[1,a]
D. pulex	Well	1	0.07 (0.01-0.15)	0.05 (0.03-0.09)
D. pulex	Well	2	0.11 (0.05-0.15)	0.10 (0.07-0.12)[b]
P. promelas	Well[x]	1	0.10 (NC)	0.10 (0.07-0.17)[c]
P. promelas	Well[y]	1	0.08 (NC)	0.09 (0.07-0.14)[d]

NC - Not Calculable; x = 48 h; y = 96 h

NaPCP is evident by the fact that its upper 95% CIs were an order of magnitude below the lower 95% CIs of the invertebrates in all but one experiment. The different water ypes did not influence the toxicity of NaPCP to a specific species. No significant differences in LC50s for duplicate experiments were observed for any species, indicating good reproducibility between all tests.

Table 4 presents Cd acute toxicity data from this and other studies. Experimental conditions were similar in all tests. Reconstituted and natural waters were used. LC50s represent measured and nominal concentrations. Methods used to calculate LC50s were different between some studies. The widest range of LC50s obtained in both water types as determined by the binomial and probit procedures are presented from this study.

Limited data are available on the toxicity of Cd to *C. reticulata*. However, Cd LC50s to *C. reticulata* differ by less than a factor of two for the studies presented. LC50s of Cd to *D. magna* cover over an order of magnitude for the six studies cited. However, if the lowest LC50 of Cd to *D. magna* is omitted from the Lewis and Weber (1985) study, the range of LC50s would be 0.012-0.043 mg/L. This results in an order of magnitude approximation of the Cd LC50 to *D. magna* (∿0.01-0.12 mg/L) for all studies. The range of Cd LC50s to *D. pulex* is smaller than that for *D. magna* with less than a

Table 3. Results of acute toxicity tests with NaPCP. The same letters on data points indicate statistically significant differences between LC50 values of different species tested in the same water type. LC50 data for invertebrates are for 48 h tests; LC50 data for *P. promelas* are 48- or 96-h tests as indicated.

Organism	Water Type	Exp. No.	LC50 (95% Confidence intervals) mg/L Binomial	Probit
C. reticulata	Recon	1	0.31 (0.20-0.60)	-
C. reticulata	Recon	2	0.44 (0.20-0.60)	0.38(0.29-0.47)
D. magna	Recon	1	1.04 (0.60-1.80)	-
*D. magna**	Recon	2	0.90 (0.60-1.80)	-
D. pulex	Recon	1	1.04 (0.60-1.80)	-
D. pulex	Recon	2	1.14 (0.60-1.80)	1.05(0-infinity)
C. reticulata	Well	1	0.28 (0.05-0.60)	0.22(0.13-0.37)[a]
C. reticulata	Well	2	0.55 (0.40-0.80)	-
D. magna	Well	1	0.49 (0.20-1.80)	0.47(0.30-0.74)[b]
*D. magna**	Well	2	1.12 (0.60-1.80)	-
*D. pulex**	Well	1	1.08 (0.60-1.80)	-
P. promelas	Well[x]	1	0.02 (0.015-0.03)**	0.02(0.018-0.025)[a,b]
P. promelas	Well[y]	1	0.02 (0.015-0.03)**	-

* - N = 5 or 6 per exposure rather than 10.
** - *P. promelas* were significantly more sensitive to NaPCP than all invertebrates in well water as determined by the binomial method.
x - 48 h
y - 96 h

four-fold difference between LC50s reported for all studies. The data on Cd acute toxicity to *P. promelas* indicate greater than an order of magnitude difference between LC50s obtained in different studies. However, the variability of Cd toxicity tests with *P. promelas* cannot be adequately asessed from these limited data. The data in Table 4 indicate that an order of magnitude approximation of LC50s of a particular toxicant to the three cladoceran species can be obtained by different investigators. These data also support the results of this study which indicate that the four test species are similarly sensitive to Cd under the test conditions employed and that differences in dilution water do not markedly effect the toxicity of Cd.

Results of acute toxicity tests with NaPCP and PCP from this and other studies are presented in Table 5. The acute toxicity of NaPCP and PCP are directly comparable because: 1) pentachlorophenol is expected to be the toxic constituent of NaPCP; 2) sodium makes up <8% ($^{w}/w$) of the NaPCP molecule and should not contribute to its toxicity and 3) a comparison of the acute

Table 4. Comparison of Cd Acute Toxicity Data.

Organism	Test Type* Duration (h), Toxicant	Hardness (mg/L $CaCO_3$)	LC50 (mg/L Cd) or Range of LC50s (n)**	References
C. reticulata	S, 48, $CdCl_2$	120 ± 10 or 200 ± 10	0.07 - 0.11 (3)	This study
C. reticulata	S, 48, $CdCl_2$	45	0.066(1)	Mount & Norberg 1984
D. magna	S, 48, $CdCl_2$	120 ± 10 or 200 ± 10	0.02 - 0.08 (4)	This study
D. magna	S, 48, $CdCl_2$	170 ± 10	0.0061 - 0.043 (7)	Lewis & Weber 1985[a]
D. magna	S, 48, $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$	45	0.065 (1)	Biesenger & Christenson 1972
D. magna	S, 48, $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$	51 to 209	0.0099 - 0.063 (5)	Chapman 1980
D. magna	S, 48, $CdCl_2$	45	0.118 (1)	Mount & Norberg 1984
D. magna	S, 48, $CdCl_2$	26 to 32	0.024 - 0.04 (3)	Schuytema et al. 1983
D. pulex	S, 48, $CdCl_2$	120 ± 10 or 200 ± 10	0.05 - 0.11 (4)	This study
D. pulex	S, 48, $CdCl_2$	170 ± 10	0.10 - 0.18 (6)	Lewis & Weber 1985[a]
D. pulex	S, 48, $CdCl_2$	45	0.068 (1)	Mount & Norberg 1984
D. pulex	S, 48, $CdNO_2$	-	0.14 - 0.15 (2)	Canton & Adema 1978
P. promelas	S, 96, $CdCl_2$	120 ± 10 or 200 ± 10	0.08 - >0.15 (2)	This study
P. promelas	S, 96, $CdCl_2$	-	0.011 - 0.141 (9)	U.S. EPA Unpublished
P. promelas	S, 96, $CdCl_2$	39 - 48	0.012 - 0.054 (6)	Spehar 1982

* S = static test; ** n = number of experiments; [a] Data for organisms unfed during tests.

Table 5. Comparison of acute toxicity data using NaPCP and PCP as the test toxicant.

Organism	Test Type* Duration (h), Toxicant	Hardness (mg/L $CaCO_3$)	LC50 (mg/L NaPCP or PCP) or Range of LC50s (n)**	References
C. reticulata	S, 48, NaPCP	120 ± 10 or 200 ± 10	0.22 - 0.55 (4)	This study
C. reticulata	S, 48, PCP	45	0.164 (1)	Mount & Norberg 1984
D. magna	S, 48, NaPCP	120 ± 10 or 200 ± 10	0.47 - 1.12 (4)	This study
D. magna	S, 48, NaPCP	170 ± 10	0.51 - 0.84 (3)	Lewis & Weber 1985[a]
D. magna	S, SR, or FT, 48, NaPCP	"Hard"	1.05***	Adema & Vink 1981
D. magna	S, 48, PCP	-	0.24 - 0.80 (6)	Canton & Adema 1978
D. magna	S, 48, NaPCP	-	0.60 (1)	Adema 1978[a]
D. magna	S, 48, PCP	45	0.143 (1)	Mount & Norberg 1984
D. pulex	S, 48, NaPCP	120 ± 10 or 200	1.04 - 1.14 (3)	This study
D. pulex	S, 48, NaPCP	170 ± 10	0.35 - 0.68 (4)	Lewis & Weber 1985[a]
D. pulex	S, 48, PCP	-	2.0 (2)	Canton & Adema 1978
D. pulex	S, 48, PCP	45	0.246 (1)	Mount & Norberg 1984
P. promelas	S, 96, NaPCP	200 ± 10	0.02 (1)	This study
P. promelas	S, 96, NaPCP	-	0.08 - 0.19 (12)	U.S. EPA unpublished
P. promelas	FT, 96, NaPCP	220	0.198 (1)	Adelman & Smith 1976

* S = Static Test, SR = Static Renewal, FT = Flow-through
** n = number of experiments.
*** LC50 is mean of two to five tests.
a Represents only data for organisms unfed during tests.

(96 h) toxicity of NaPCP and PCP to four species of freshwater fish revealed no statistical differences in LC50s of the two chemicals (Johnson and Finley 1980). The same considerations discussed for the Cd data apply to these data. The widest range of LC50s for each species in this study is again presented.

The LC50s to C. reticulata were similar when comparing this study and the Mount and Norberg (1984) investigation. Maximum differences in LC50s between these studies were within a factor of four. Good agreement between LC50s of NaPCP and PCP to D. magna is seen for the six studies cited. There is less than an eight-fold difference for all LC50s reported for D magna. Similar reproducibility between investigators was observed for D. pulex, with the largest discrepency in LC50s differing by approximately a factor of eight. All LC50s of NaPCP to P. promelas are within a range of an order of magnitude with the animals used in this study being most sensitive. However, when viewing all data, it appears that a more representative estimate of the LC50 to P. promelas lies within the range presented for the other studies. NaPCP and PCP LC50s were similar for each of the invertebrates, supporting the findings of Johnson and Finley (1980) which indicated similar toxicity of each chemical to fish. Inter-species sensitivities were similar for the invertebrates, supporting the results of this study. The data in Table 5 also indicate that differences in dilution waters used in these studies do not markedly influence the results of acute toxicity tests with NaPCP and PCP.

Conclusions concerning the objectives of this study are as follows: 1) the four species were similarly sensitive to Cd under the conditions employed; 2) the invertebrates were similar in their sensitivity to NaPCP or PCP but data for P. promelas were too limited to draw a conclusion as to the relative sensitivity of this species; 3) estimates of LC50s within a range of an order of magnitude are attainable for these chemicals and test organisms in studies using similar test conditions and 4) differences in dilution waters used in this study did not influence the results of acute toxicity tests with Cd or NaPCP.

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