Environmental Engineering

Department of Civil Engineering
University of Massachusetts at Amherst

University of Massachusetts
Amherst, Massachusetts 01003

Department of Civil Engineering
Environmental Engineering Program

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Neil M. Ram, James Williams and Kevin C. Sheehan

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Variability in LC50 Values Determined by Flow-through Vs. Static Fish Toxicity Testing Procedures

by

Neil M. Ram, PhD
Assistant Professor

James Williams
Graduate Research Assistant

and

Kevin C. Sheehan
Research Engineer

Department of Civil Engineering
Environmental Engineering Program
University of Massachusetts
Amherst, Massachusetts 01003

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As previously described in Environmental Engineering Publications 72-83-3 and 73-83-2, acute toxicity tests can be conducted using either static (S) or flow-through (Ft) procedures. The static toxicity test is usually considered to be suitable in evaluating compounds which are highly stable and whose toxicity is not associated with high oxygen demand, while flow-through tests are generally appropriate when testing unstable substances or compounds having high BOD's.

The purpose of this report is to review the degree to which these two methods of determining toxicity give rise to different LC50 values. The flow-through procedure has merit since it approximates natural stream conditions more closely than the static bioassay procedure. However, the complexity and cost of apparatus required for the flow-through test may over-ride any advantage it has. The static test, on the other hand, has basic, inexpensive supply requirements. If there does not appear to be a significant difference or any consistent trend in the data generated with the two techniques, the static test may prove preferable due to its simplicity.

Tables 1 through 8 list 96 hour LC50 values for several compounds which have been tested under both static and flow-through conditions. For metals, there does not appear to be any significant trend in terms of the relative magnitudes of the LC50 values generated with flow-through and static toxicity tests. Copper, for example, did not display any dramatic difference under the two test conditions, while nickel showed somewhat greater toxicity under flow-through conditions. The results for zinc were generally erratic, but the average of the data showed greater toxicity under flow-through conditions.

Several observations can be made with respect to the acute toxicity of organic compounds to fish under flow-through vs. static conditions. Table 6 indicates that comparative acute toxicity data for different organisms under flow-through vs. static conditions varies both with the particular compound as well as with the test organism. The relative flow-through:static toxicity of pyrethrum, for example, was 0.59 for Coho Salmon and 2.1 for Bluegills. RU-11679 displayed greater toxicity to Coho Salmon and Steelhead Trout in flow-through tests, but was more toxic to channel catfish in static tests. All of the remaining compounds shown in Table 6 displayed consistently greater toxicity under flow-through conditions.

Table 7 illustrates the acute toxicity of a variety of compounds including two insecticides, a lampracide, a wetting agent, and a metal to several fish. Of the two insecticides reported, Malthion proved more toxic under static conditions, while Endrin was more toxic under flow-through conditions. Excluding nickel, all other compounds listed in Table 7 were as or more toxic under static test conditions.

These data indicate that the relative toxicity of a test compound under flow-through vs static test conditions is somewhat variable. As discussed previously, there may be circumstances (excessive oxygen demand, high volalitity) which require use of flow-through test conditions. However, under most circumstances it is our recommendation that static bioassays be conducted over flow-through studies, owing to their lower costs, sampling requirements, and personnel needs, and the corresponding valuable toxicity data obtained from such studies.

Table 1

Toxicity of Copper Sulfate to the Fathead Minnow at Various Levels of Hardness Under Static (S) and Flow-through (Ft) Test Conditions

Test Type	Hardness (mg/L as $CaCO_3$)	LC50 (µg/L) (Expected)[1]	LC50 (µg/L) Observed	Reference
Ft	31	83	75	Mount and Stephan (1969)
Ft	46	120	89	Lind
Ft	45	118	121	Lind
Ft	48	125	114	Lind
Ft	200	479	440	Geckler 1976
Ft	200	479	490	Geckler 1976
Ft	202	483	460	Pickering 1977
Ft	202	483	490	Pickering 1977
S	20	55	23	Pickering 1977
S	31	83	84	Mount and Stephan 1969
S	200	478	430	Mount 1968
S	360	832	1450	Pickering and Henderson 1966

1. Expected LC50 values were calculated from the equation:
   $\text{toxicity} = e^{[.94(\ln \text{hardness}) + 1.19]}$ (Ambient water quality criteria for copper, 1980)

Table 2

Mean Flow-through (Ft) Vs. Static (S) Toxicity Values of Copper Sulfate to Fathead Minnows[1]

Hardness (mg/L as $CaCO_3$)	Observed Toxicity (LC50) (μg/L)						
	Flow-through			Static			Ft/S
	Value	n	σ	Value	n	σ	
31	75	1	-	84	1	-	0.89
200 or 202[2]	470	4	21.21	430	1	-	1.09

1. This data is based on values presented in Table 1. The pH, DO, and temperature were relatively consistent between the different studies, and have been shown not to significantly effect toxicity over narrow ranges.

2. In four different experiments conducted in waters with hardnesses of 202 mg/L and 200 mg/L as $CaCO_3$.

Table 3

Toxicity of Zinc Sulfate to the Fathead Minnow in Waters of Varying Hardness Under Flow-through (Ft) and Static (S) Conditions

Test Type	Hardness (mg/L as $CaCo_3$)	Expected LC50[1] (mg/L)	Observed LC50 (mg/L)	References
Ft	46	4.06	0.6	Benoint and Holcombe, 1966
Ft	50	4.35	12.5	Mount, 1966
Ft	50	4.35	13.8	Mount, 1966
Ft	50	4.35	13.7	Mount, 1966
Ft	50	4.35	6.2	Mount, 1966
Ft	200	13.73	29.0	Mount, 1966
Ft	200	13.73	8.2	Mount, 1966
Ft	203	13.90	8.4	Brungs, 1969
Ft	203	13.90	10.0	Brungs, 1969
S	45	3.98	3.1	Judy and Davies, 1979
S	166	11.76	7.6	Rachlin and Perlmutter, 1968
S	203	13.90	12.0	Brungs, 1969
S	203	13.90	13.0	Brungs, 1969
S	360	22.37	33.4	Pickering and Henderson, 1966

1. Expected LC50 values were calculated from the equation: toxicity = $e^{[0.83(\ln \text{hardness}) + 5.13]}$ (Ambient Water Quality Criteria for Zinc, 1980).

Table 4

Mean Flow-through (F) Vs. Static (S) Toxicity Values of Zinc Sulfate to the Fathead Minnow[1]

Hardness (mg/L as $CaCO_3$)	Mean Toxicity (LC50) (mg/L) Flow-through value	n	σ	Static value	n	σ	Ft/S
203	9.20	2	0.8	12.50	2	0.5	.74
46	0.60	1	–	–	–	–	.19
45	–	–	–	3.10	1	–	

1. This data is based on values presented in Table 3.

Table 5

Flow-through (Ft) Vs. Static (S) Toxicity Values of Phenol to Fathead Minnows

Test Type	Hardness[1] (mg/L as $CaCO_3$)	LC50 (mg/L)	Reference
Ft	-	67.5	Ambient Water Quality Criteria, Phenol, 1980
Ft[2]	-	36.0	Ruesink and Smith, 1975
Ft[2]	-	24.0	Ruesink and Smith, 1975
Ft	-	28.8	Phipps, et al.
S	20	34.3	Pickering and Henderson, 1966
S	360	32.0	Pickering and Henderson, 1966
S	-	32.0	Mattson, et al., 1976
Mean Ft/S		1.19	
Mean Ft/S[3]		0.90	

1. Variations in hardness had no apparent effect on toxicity of phenol.

2. Total alkalinity = 218-230 (mg/L as $CaCO_3$); pH = 8.0.

3. Excluding flow-through LC50 value equal to 67.5.

Table 6

Toxicity of Pyrethrum Extract and Five Pyrethroids to Fish in Static (S) and Flow-through (Ft) tests at 12°C (March, 1976)[1]

		96-hr LC50 (ug/L)[2]		
Formulation	Species	Static	Flow-through	Flow-through/ Static
Pyrethrum (20%)	Coho Salmon	39.0 (33.1-46.0)	23.0 (17.8-29.7)	.590
	Steelhead trout	24.6 (20.4-29.6)	22.5 (19.2-26.3)	.915
	Channel Catfish	114 (95.0-1.37)	132 (117-149)	1.158
	Bluegill	49.0 (39.2-61.3)	104 (80.3-135.0)	2.122
	Yellow Perch	50	44.5 (36.4-54.3)	.888
Dimethrin (96%)	Channel Catfish	1140 (1020-1280)	165 (126-126)	.145
	Bluegill	37.5 (28.1-50.0)	22.3 (16.7-29.5)	.595
d-transallethrin (90%)	Coho Salmon	22.2 (20.6-23.9)	9.4 (7.91-11.2)	.423
	Steelhead Trout	17.5 (13.1-23.4)	9.7 (8.0-11.6)	.554
	Channel Catfish	30.2	27.0	.894
Ru-11679 (95%)	Coho Salmon	.635 (.580-.696)	.151 (.132-.173)	.238

Table 6, continued

Formulation	Species	96-hr LC50 (ug/L)[2] Static	Flow-through	Flow-through/Static
	Steelhead Trout	.110 (.091-.134)	.100 (.075-.1330	.909
	Channel Catfish	.630 (.402-.853)	.700 (.583-.840)	1.111
S-bioallethrin (98%)	Fathead Minnow	80.0 (65.9-97.1)	53.0 (35.9-78.3)	.663
SBP-1382 (89%)	Coho Salmon	1.510	.276	.183
		.450	.275	.611
	Bluegill	2.62 (2.25-3.19)	.275 (.237-.319)	.105
	Yellow perch	2.36 (1.96-2.84)	.513 (.441-.597)	.217

1. Hardness for all of the various tests was reported to be between 280 and 320 mg/L as $CaCO_3$.

2. 95% confidence limits are shown in parentheses.

3. For N = 18 organisms and formulations tested:

   Ft/S = .523, .685, and .489 as the geometric mean, arithmetic mean and standard deviation, respectively.

Table 7

Quotients of 96-hr LC50 Values of Flow-through (Ft) and Static (S) Tests Using Bluegill Sunfish and Fathead Minnows as Test Organisms

Toxicant	Species	Hardness (mg/L as $CaCO_3$)	LC50 (µg/L) Ft	Static	Ft/S	Reference
Tergitol 15-S-9	Bluegill	40-48	4.80	4.60	1.04	Krzeminski,1975
Neodol 25-9	Bluegill	40-48	2.10	2.10	1.0	Krzeminski,1975
TFM	-	-	3.28	2.85	1.19	U.S.Wildlife and Fish,1975
TFM	-	-	3.42	2.75	1.24	U.S.Wildlife and Fish,1975
TFM	-	-	2.15	1.80	1.19	U.S.Wildlife and Fish,1975
Nickel	Fathead minnow	-	28.00	32.00	0.88	Pickering,1974
Nickel	Fathead minnow	-	25.00	27.00	0.93	Pickering,1974
Malathion	Bluegill	200	10.45	9.00	1.16	Eaton,1970
Endrin	Bluegill	116	0.39	0.77	0.51	Lincer,1970

Ft/S Geometric Mean = 0.98

Ft/S Arithmetic Mean = 1.016

Standard Deviation
of Arithmetic mean = 0.288

n = 9

Table 8

Toxicity of Endrin to Fathead Minnows Under Flow-through (Ft) and Static (S) Conditions

Test Type	LC50 (μg/L)	Hardness (mg/L as $CaCO_3$)	Reference
Ft	0.50	149	Brungs and Bailey, 1966
Ft	0.49	149	Brungs and Bailey, 1966
Ft	0.40	149	Brungs and Bailey, 1966
Ft	0.45	300	Brungs and Bailey, 1976
Ft	0.26	149	Brungs and Bailey, 1976
S	1.10	20	Henderson, et al., 1959
S	1.40	400	Henderson, et al., 1959

1. Hardness had no significant effect on toxicity values reported for flow-through conditions.

2. Mean Ft-LC50 = 0.42; n = 5; σ = 0.087
   Mean S-LC50 = 1.250; n = 2; σ = 0.15
   Mean Ft/S = 0.336

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