Bull. Environ. Contam. Toxicol. (1992) 49:361–367

Environmental Contamination and Toxicology

New Rapid Method to Evaluate the Median Effect Concentrations of Xenobiotics in Hydrobionts

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Common methods for evaluating the median lethal concentration of xenobiotics in hydrobionts are usually time-intensive, demand a large number of test animals, and typically use large quantities of testing compounds (Litchfield and Wilcoxon, 1949; Belen'kiy, 1963). There are some rapid and simpler methods to evaluate an LC_{50} (Van der Varden, 1960; Prozorovsky et al., 1978; Deichmann and Le Blanc, 1943). However, these methods also have some limitations. For instance, Van der Varden's method is reliable only when the doses (concentrations) under evaluation are close to the true median dose. Moreover, this method requires that the exact angle coefficient of the slope be known. This information may not be known for newly synthesized compounds (Deichmann and LeBlanc, 1943). The method discussed by Prozorovsky and coauthors has limitations related to the impossibility of evaluating certain sequelae of toxic responses (Prozorovsky et al., 1978). The method of Deichmann and Le Blanc is useful only for an approximate evaluation of the LC_{50} for highly toxic compounds. To date, the assessment of the toxicity of chemicals has utilized the approach for which multiple concentrations are simultaneously tested. In a rapid assessment approach, it may be desirable to quickly approximate the LC_{50}, using fewer organisms and a small amount of toxicant. This paper proposes the use of one concentration of a chemical, and one group of animals in a rapid analytical method of "one-point" to evaluate the LC_{50} (or any median effective concentrations, EC_{50}) of toxic compounds in hydrobionts of different taxa. This recently proposed method of Frumin (1991), which uses a "one-point" measurement approach for calculating an LD_{50}, is based on the Gauss normal distribution law. This method has not been evaluated for hydrobionts although it has been applied to a variety of xenobiotics in mammals.

MATERIALS AND METHODS

As test-objects, the following animals were used. Adult specimens of tubificid worms *Tubifex sp.*, (*Olygochaeta, Tubificidae*), freshwater snails *Lymnaea stagnalis* and *Planorbis corneus* (*Mollusca; Gastropoda*), *Asellus aquaticus* (*Crustacea; Isopoda*), midge larvae *Chironomus riparius* (*Diptera; Chironomidae*), common carp young-of-year (YOY) *Cyprinus carpio* (*Pisces; Cyprinidae*), bream YOY *Abramis brama* (*Pisces; Cyprinidae*), perch YOY *Perca fluviatilis* (*Pisces; Percidae*), and mozambique tilapia *Oreochromis mossambicus* (*Pisces; Cichlidae*) 4 to 5 months old. The experiments were conducted using static-replacement of water once every 24 hours, following accepted methods (U.S. Environmental Protection Agency, 1975). All test concentrations and controls were performed

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using well water for media preparation. Standard water characteristics were as follows: Ca++ content 40 mg/l, oxygen content 6.5 to 8.0 mg/l, pH 7.8 to 8.1 and temperature from 17 to 21 C (22 - 24 C in the experiments with tilapia). The experiments were carried out in glass or plexiglas aquaria with volumes ranging from 100 ml to 30 l, depending on the size of the experimental animals. The following chemicals were tested using both the typical approach of LC_{50} determination and the "one-point" method: DDVP (Dichlorvos, 97 % active ingredient), Chlorophos (Dipterex, 80 % technical preparation), Carbophos (Malathion, 40 % emulsion) (all organophosphorus insecticides), polyaromatic hydrocarbon naphthalene (technical grade), and cadmium sulfate (reagent grade). Nominal toxicant concentrations were calculated based upon the percentage active ingredient; cadmium concentration was based on the Cd++ ion concentration. Acetone was used as the carrier solvent for the pesticides and naphthalene. Cadmium sulfate was predissolved in distilled water. The solvent concentrations in test waters did not exceed 0.01 % of volume. The LC_{50} values were calculated following Litchfield and Wilcoxon (1949) and compared with the values calculated following Frumin's (1990) "one-point" method. For these LC_{50} calculations, we propose to use the following equation:

$$LC_{50} = LCp / (1 + 0.2 K) \qquad (1)$$

The terms in the equation (1) are: LCp is the concentration at which an effect with a given probability, p, is observed. The coefficient "K" is a normalized deviation (ND); the coefficients are presented in Table 1. The ND is dependent on the number of

Table 1. Values of the coefficient k in the equation (1). Number of animals in which any desirable effect was evident

Number of animals in group	1	2	3	4	5	6	7	8	9
5	-0.842	-0.250	0.250	0.842					
6	-0.967	-0.428	0.000	0.428	0.967				
7	-1.067	-0.565	-0.180	0.180	0.565	1.067			
8	-1.151	-0.680	-0.319	0.000	0.319	0.680	1.151		
9	-1.216	-0.764	-0.432	-0.139	0.139	0.432	0.764	1.216	
10	-1.290	-0.850	-0.530	-0.260	0.000	0.260	0.530	0.850	1.290

test animals and the proportion of test animals affected by the toxicant. If more than 10 animals are used in an experimental group, "K" is determined using, for instance, normalized distribution tables, such as found in Bolshev and Smirnov (1983), where "K" values are given for specific normal distribution functions. For calculating LC_{50} standard error values (m), we propose the following equation:

$$m = 0.2\ LC_{50} / \sqrt{n} \qquad (2)$$

where "n" is the number of test animals in the experimental group. Confidence intervals for an LC_{50} may be calculated using Student's

Table 2. **Median lethal concentrations values been evaluated by the comparing probit analysis method and by the proposed rapid one-point method.**

Species	Toxicant	Duration (hrs)	Toxicant concentration (mg/l)	Effect died/N[a]	LC_{50} (mg/l) probit analysis	LC_{50} (mg/l) proposed rapid method	
carp	chlorophos	48	275	1/6	417.5[b]	340.9	(269.4-412.4)
			325	2/6	(303.3-531.7)	355.4	(280.0-430.0)
			425	3/6		425.0	(335.8-514.2)
carp	DDVP	48	18	1/6	21.9	22.3	(17.6-27.0)
			20	2/6	(20.4-23.4)	21.9	(17.3-26.5)
			22	3/6		22.0	(17.4-26.6)
			24	4/6		22.1	(17.5-26.7)
			26	5/6		21.8	(17.2-26.4)
bream	DDVP	48	27	2/6	28.1	29.5	(23.3-35.7)
			29	4/6	(27.1-29.1)	26.7	(21.1-32.3)
			31	5/6		26.0	(20.5-31.5)
perch	chlorophos	48	0.40	1/8	0.67	0.52	(0.43-0.61)
			0.56	4/8	(0.52-0.82)	0.56	(0.47-0.65)
			0.64	5/8		0.60	(0.50-0.70)
			0.72	3/8		0.77	(0.64-0.90)
tilapia	DDVP	24	10.0	3/10	12.8	11.2	(9.6 -12.8)
			12.5	3/10	(11.2-14.4)	14.0	(12.0-16.0)
			15.0	7/10		13.6	(11.7-15.5)
			17.5	9/10		13.9	(11.9-15.9)
bream	Cd++	24	5.0	2/10	6.1	6.0	(5.1-6.9)
			6.5	5/10	(3.9-8.3)	6.5	(5.6-7.4)
			8.0	7/10		7.2	(6.2-8.2)
			11.0	9/10		8.7	(7.4-10.0)
			17.0	8/10		14.5	(12.4-16.6)

Table 2. (Continued) **Median lethal concentrations values been evaluated by the comparing probit analysis method and by the proposed rapid one-point method.**

Species	Toxicant	Duration (hrs)	Toxicant concentration (mg/l)	Effect died/N[a]	LC_{50} (mg/l) probit analysis	LC_{50} (mg/l) proposed rapid method	
tilapia	Cd++	24	140.0	3/10	149.8	156.6	(134.2-178.0)
			145.0	3/10	(110.1-203.8)	162.2	(139.0-185.4)
			150.0	5/10		150.0	(128.6-171.4)
			155.0	7/10		140.1	(120.0-160.1)
			160.0	7/10		144.7	(124.0-165.4)
bream	naphthalene	24	6.0	1/10	10.0	8.1	(6.9-9.3)
			9.0	3/10	(9.1-10.9)	10.1	(8.7-11.5)
			9.5	5/10		9.5	(8.1-10.9)
			10.5	6/10		10.0	(8.6-11.4)
			12.0	6/10		11.4	(9.8-13.0)
tilapia	naphthalene	24	21.0	3/10	22.4	23.5	(20.1-26.9)
			21.5	2/10	(17.4-28.8)	25.9	(22.2-29.6)
			22.0	4/10		23.2	(20.0-26.6)
			22.5	5/10		22.5	(19.3-25.7)
			23.0	7/10		20.8	(17.8-23.8)
			23.5	7/10		21.2	(18.2-24.2)
Tubifex sp.	chlorophos	48	100.0	2/30	236.6	143.7	(133.0-154.4)
			150.0	5/30	(127.0-440.9)	186.1	(172.2-200.0)
			200.0	12/30		210.5	(194.7-226.3)
			250.0	14/30		254.6	(235.5-273.7)
			300.0	21/30		271.7	(251.4-292.0)
Tubifex sp.	carbophos	48	30.0	2/30	44.7	43.1	(39.9-46.3)
			40.0	6/30	(29.8-67.1)	48.1	(44.5-51.7)
			50.0	22/30		44.4	(41.1-47.7)
			60.0	27/30		47.8	(44.2-51.4)

Table 2. (Continued) **Median lethal concentrations values been evaluated by the comparing probit analysis method and by the proposed rapid one-point method.**

Species	Toxicant	Duration (hrs)	Toxicant concentration (mg/l)	Effect died/N[a]	LC_{50} (mg/l) probit analysis	LC_{50} (mg/l) proposed rapid method	
Tubifex sp.	DDVP	48	50.0	2/30	130.9	71.8	(69.2-74.4)
			100.0	8/30	(74.0-231.8)	114.4	(105.8-123.0)
			150.0	16/30		147.3	(136.3-158.3)
			200.0	24/30		171.2	(158.4-184.0)
Asellus	chlorophos	48	400.0	4/24	572.8	496.3	(454.4-538.2)
			500.0	8/24	(417.3-786.3)	547.1	(500.9-593.3)
			600.0	12/24		600.0	(549.3-650.7)
			700.0	18/24		617.3	(565.1-669.5)
Asellus	carbophos	48	25.0	3/22	60.0	32.1	(29.3-34.9)
			50.0	11/22	(38.9-92.7)	50.0	(45.6-54.4)
			75.0	13/22		71.7	(65.3-78.1)
			100.0	16/22		89.3	(81.4-97.2)
Asellus	DDVP	48	100.0	4/22	127.4	122.5	(111.6-133.4)
			125.0	9/22	(90.2-180.3)	131.0	(119.4-142.6)
			150.0	16/22		133.9	(122.0-145.8)
			175.0	21/22		129.6	(118.1-141.1)
Lymnaea	carbophos	48	5.0	1/18	13.3	7.4	(6.7 - 8.1)
			10.0	3/18	(5.5-32.0)	12.4	(11.2-13.6)
			15.0	10/18		14.6	(13.1-16.1)
			20.0	15/18		16.8	(15.1-18.4)
Planorbis	chlorophos	48	250.00	5/20	307.6	288.7	(261.7-315.7)
			300.0	9/20	(215.7-439.5)	308.0	(279.2-336.8)
			350.0	13/20		324.7	(294.4-355.0)
			400.0	17/20		331.1	(300.2-362.0)

Table 2. (Continued) **Median lethal concentrations values been evaluated by the comparing probit analysis method and by the proposed rapid one-point method.**

Species	Toxicant	Duration (hrs)	Toxicant concentration (mg/l)	Effect died/N[a]	LC_{50} (mg/l) probit analysis	LC_{50} (mg/l) proposed rapid method	
Planorbis	carbophos	48	50.0	4/24	81.3	62.0	(56.3-67.7)
			75.0	8/24	(33.6 -196.8)	82.1	(75.2-89.0)
			125.0	20/24		104.7	(95.9-113.5)
			150.0	23/24		111.1	(101.7-120.5)
Planorbis	DDVP	48	50.0	2/20	72.9	67.2	(60.9-73.5)
			75.0	10/20	(38.7 -137.4)	75.0	(68.0-82.0)
			100.0	15/20		88.2	(80.0-96.4)
			125.0	18/20		99.5	(90.2-108.8)
Chironomus	chlorophos	48	700.0	4/24	968.3	868.5	(795.1-941.9)
			900.0	10/24	(829.4-1129.8)	939.5	(860.1-1018.9)
			1100.0	15/24		1175.2	(1075.9-1274.5)
			1300.0	20/24		1088.8	(996.8-1180.8)
			1500.0	21/24		1219.5	(1116.4-1322.6)
Chironomus	carbophos	48	10	2/17	26.9	13.2	(11.8-14.6)
			20	7/17	(20.0-36.2)	21.0	(18.8-23.2)
			40	11/17		37.2	(33.4-41.0)
			60	14/17		50.6	(45.4-55.8)
Chironomus	DDVP	48	150	6/24	230.1	173.2	(158.6-187.8)
			200	7/24	(161.6-328.0)	224.7	(205.7-243.7)
			250	11/24		255.1	(233.5-276.7)
			300	18/24		264.6	(242.2-287.0)
			350	19/24		301.2	(275.7-326.7)

[a] = N = number of animals tested
[b] = mean (95% confidence interval)

coefficients (t) for P = 0.05 and f = n - 1. The equation is as follows:

$$LC_{50} \pm mt = LCp\,/\,(1 + 0.2\,K) \pm 0.2\,LC_{50}\,t\,/\sqrt{n} \qquad (3)$$

These equations as well as the method itself were described in more detail in Frumin (1991).

RESULTS AND DISCUSSION

The comparative LC_{50} values of toxicants tested by both methods are listed in Table 2. These data show that in 25 % of the tests, the values from both methods are essentially equivalent. Twenty-nine percent of the tests yielded differences of 5 to 10 %, 21 % showed differences from 10 to 20 %, and the remaining 25 % yielded differences exceeding 20 %. In any case, the differences between the two methods in calculating an LC_{50} were within 2.5 times the LC_{50} estimates; this degree of similarity in test results is considered within the range of acceptable variability in static toxicity tests. Thus, this proposed method allows one to rapidly determine an effect concentration to within at least the same order of those tests using multiple concentrations. The results of our study show that by using the proposed express method of "one point", one may quickly evaluate both the median lethal (effect) concentrations, with standard error, and confidence intervals. The benefits of using this method are that a minimal number of test animals and lesser quantities of chemicals are required.

Acknowledgements. We thank Dr. Thomas LaPoint for his assistance in editing the initial translation into English and Mr. Richard W. Carlson for reviewing the completed manuscript.

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Received October 28, 1991; accepted March 20, 1992.