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Comparison of Ethanol Toxicity to *Daphnia magna* and *Ceriodaphnia dubia* Tested at Two Different Temperatures: Static Acute Toxicity Test Results

I. T. Takahashi,† U. M. Cowgill, and P. G. Murphy

Health and Environmental Sciences, 1702 Building, The Dow Chemical Company, Midland, Michigan 48674

Ethanol is a commonly used solvent in toxicity testing, yet there are few studies in the literature devoted to its toxicity to zooplankton (Loeb 1909; Barera and Adams 1983). The purpose of this study was to compare the response of Daphnia magna Straus 1820 and Ceriodaphnia dubia J. Richard 1894 (Berner 1986) to ethanol. Two temperatures were selected because most toxicity data involving D. magna has been carried out at 20°C while all discussions concerning C. dubia appear to relate to temperatures oscillating around 24°C. Thus, the response of these two organisms to ethanol was examined at 20°C and at 24°C.

MATERIALS AND METHODS

C. dubia and D. magna were mass cultured and acclimated to each of two temperatures, 20°C and 24°C, for a period of at least 10 weeks. C. dubia was maintained in filtered (0.22 um), autoclaved Lake Huron water; that used for D. magna was Lake Huron water, adjusted to a hardness of about 170 mg L^{-1} (calculated as $CaCO_3$), autoclaved and aerated for 24 h prior to use. The mean chemical analyses of these waters used to sustain these two cladocerans for the period of the study are set forth in Table 1. At the time this study was completed D. magna had been cultured in adjusted, autoclaved and aerated Lake Huron water for three years, whereas C. dubia had been in residence only four months. C. dubia was sustained on algae in an axenic environment. This cladoceran was received under sterile conditions but the animals themselves were not bacteria-free.

Both D. magna and C. dubia were maintained in environmental growth chambers devoted to rearing and two such chambers were employed, one adjusted to about 20°C and the other to about 24°C. Neonates for testing were gathered by isolating gravid females in appropriate vessels 12 h prior to setting tests and subsequently separating the two age classes using suitably sized sieves. Culture conditions for both organisms may be found in Tables 2 and 3.

Two bacteria-free algal diets were developed to maintain the

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Send reprint requests to U. Cowgill at above address.

Table 1. Typical chemical analyses of the dilution waters (μgL^{-1}) used for culture. This represents many analyses and ranges are presented when one or more analyses were below limits of detection.

Substance Analyzed	Daphnia magna	Ceriodaphnia dubia
Aluminum	85 - ND (50)	ND (50)
Ammonia	34 - ND (5,10)	35
Arsenic	0.6	NA
Barium	15	NA
Boron	325	20
Bromine	26	NA
Calcium	46750	26800
Chloride	57000	NA
Chromium	20 - ND (5)	ND (5)
Copper	ND (5) - ND (10)	ND (5)
Fluorides	62	80
Iron	15 - ND (5,10)	20
Lead	ND (5)	ND (5)
Lithium	ND (5)	NA
Magnesium	8150	9500
Manganese	ND (1,5)	ND (5)
Molybdenum	ND (5)	NA
Phosphorus	30	NA
Potassium	1028	1000
Silicon	3480	3800
Sodium	4615	4300
Sulfur	5595	5700
Zinc	35 - ND (10)	ND (5)
Total dissolved solids	289550	140000
Total organic carbon	5520	5280

NA, not analyzed; ND ( ), not detected at level noted in parentheses. If two different levels were noted, they are so indicated

organisms. D. magna was sustained by the green alga Selenastrum capricornutum Printz reared in a medium designed by Provasoli and Pintner (1953) but with increased amounts of $NaNO_3$ and K_2HPO_4. The green alga Ankistrodesmus convolutus Corda was used to maintain C. dubia. A medium developed by Keating (1985) was employed. The selection of food and handling of C. dubia have been discussed elsewhere (Cowgill et al 1985a; 1985b).

Selenastrum capricornutum originated from the Starr collection (Starr 1978). A. convolutus was isolated from Linsley Pond, North Branford, Connecticut by K. Keating of Rutgers University, New

Jersey. D. magna was obtained from L. Provasoli of Yale University, New Haven, Connecticut and is probably British in origin. C. dubia originated from Lake Superior, Minnesota and was identified by D. Berner of Temple University, Philadelphia, Pennsylvania.

Table 2. Conditions for culture of Ceriodaphnia dubia

Variable	At 20°C	At 24°C
Temperature (°C)	20.1 ± 0.3	24.2 ± 0.3
Light intensity (lux)	645 ± 85	646 ± 85
Photoperiod (h)	16 light/8 dark	16 light/8 dark
Initial pH	8.2 ± 0.1	8.2 ± 0.1
Final pH	9.0 ± 0.6	8.8 ± 0.6
Hardness as $CaCO_3$ (mgL^{-1})	90.3 ± 4.4	90.3 ± 4.4
Alkalinity (mg $CaCO_3$ L^{-1})	70.0 ± 4.0	70.0 ± 4.0
Conductivity (umhos cm^{-1})	169.5 ± 9.5	169.5 ± 9.5
Dissolved oxygen (mgL^{-1})	8.7 ± 0.9	8.3 ± 0.2
Diet	A. convolutus	A. convolutus
Feeding density (cells mL^{-1})	30000 ± 2000	30000 ± 2000
Feeding rate (mL algae 0.2 L^{-1})	3	3
Feeding frequency	every other day	every other day
Culture vessel capacity (L)	0.2	0.2
Habitat change (7 d/week)	every other day	every other day

Table 3. Conditions for culture of Daphnia magna

Variable	At 20°C	At 24°C
Temperature (°C)	20.0 ± 1.0	24.2 ± 0.3
Light intensity (lux)	1916 ± 75	1892 ± 100
Photoperiod (h)	16 light/8 dark	16 light/8 dark
pH	8.0 ± 0.1	8.0 ± 0.1
Hardness as $CaCO_3$ (mgL^{-1})	159.6 ± 7.1	159.6 ± 7.1
Alkalinity (mg $CaCO_3$ L^{-1})	64.6 ± 6.4	64.6 ± 6.4
Conductivity (umhos cm^{-1})	319.1 ± 14.8	319.1 ± 14.8
Dissolved oxygen (mg L^{-1})	9.0 ± 0.3	8.4 ± 0.3
Diet	S. capricornutum	S. capricornutum
Feeding rate (dry equivalent of mg algae/L of daphnid culture water)	1.25	1.25
Feeding frequency	Every other day	MWF
Culture vessel capacity (L)	2	2
Habitat Change	MWF	MWF

The ethanol used in this study was pure (absolute) ethyl alcohol (dehydrated U.S.P.) and was purchased from the U.S. Industrial Chemical Company, Tuscola, Illinois. Appropriate stock solutions were prepared for each test chemical. To ensure constant concentration stability, covered vessels were utilized in all testing. Only nominal concentrations were employed since estimated loss of ethanol over the 48-h period was less than 10 percent.

The procedures followed for acute toxicity testing were those recommended by the American Society of Testing and Materials (1980). Each test consisted of exposing groups of ten neonates of D. magna and C. dubia to various concentrations of ethanol and dilution water controls. All tests at 20°C for D. magna and C. dubia and 24°C for C. dubia were carried out in triplicate while those of D. magna at 24°C were replicated six times. Each triplicate or sextuplicate series consisted of concentrations that were randomly assigned within the appropriate ranges. All test concentrations were set in triplicate, and in addition a fourth vessel was set at the high, middle, low and control concentrations for the purpose of measuring dissolved oxygen, pH and temperature. Test conditions for both organisms are enumerated in Tables 4 and 5. Mortality was confirmed by microscopic examination

Table 4. Conditions under which 48-h static acute tests with Daphnia magna were conducted.

Variable	20°C	24°C
Photoperiod (h)	16 light/8 dark	
Diet	not fed	
Test vessel (glass ml)	covered beakers	
ethanol	250	
Dilution water (ml)	200	
Test Chamber	Environmental Chamber	
Daily Observations		
Dissolved oxygen ($mg\ L^{-1}$)	8.5 ± 1.7 - 10.3 ± 1.0	7.6 ± 0.7 - 8.9 ± 0.4
pH	7.7 ± 0.2 - 8.3 ± 0.1	7.8 ± 0.1 - 8.4 ± 0.5
Temperature (°C)	20.0 ± 0.1 - 20.8 ± 0.1	23.5 ± 0.3 - 24.5 ± 0.3
Total control loss	0	0.67
Effect criteria	Mortality	Mortality

The Thompson (1947) method of moving averages was used to calculate the LC50 value and the 95% confidence interval. The LC50 value is the statistically determined concentration of the test material at which half of the test organisms die within 48 h.

RESULTS AND DISCUSSION

The LC50 data for 15 tests involving the response of two cladocerans at two temperatures to ethanol are shown in Table 6.

Table 5. Conditions under which 48-h static acute tests with Ceriodaphnia dubia were conducted. Mortality was confirmed by microscopic examination

Variable	20°C	24°C
Photoperiod (h)	16 light/8 dark	
Diet	not fed	
Test vessel (glass)	Petri dishes 60 x 15 mm	Vials 3 - 5 dram
Dilution water (ml)	10	10
Test Chamber	Environmental Chamber	
Daily Observations		
Dissolved oxygen (mg L^{-1})	8.0 ± 1.1 - 9.2 ± 0	8.4 ± 0.4 - 10.3 ± 0.1
pH	8.2 ± 0.1 - 8.3 ± 0	8.2 ± 0.1 - 8.4 ± 0.1
Temperature (°C)	20.4 ± 0.1 - 20.9 ± 0.1	24.0 ± 0.1 - 24.9 ± 0
Total control loss	6.67	7.78
Effect criteria	Mortality	

A comparison of the toxicity of ethanol to D. magna and C. dubia at 20°C and 24°C may be summarized as follows:

1. There is no statistically significant difference (>10%) by chi-square between the toxicity of ethanol to D. magna at 20°C and 24°C.

2. There is a statistically significant difference (<0.1%) between the toxicity of ethanol to C. dubia at 20°C and 24°C. A chi-square of 190.3 suggests that the two LC50 figures are different at a level of significance beyond 0.1%. This organism is more sensitive to ethanol at 24°C than at 20°C. In the absolute sense these comments are technically correct though their toxicological meaning may be insignificant.

3. C. dubia is 1.9 times more sensitive to ethanol than D. magna at 20°C and 2.4 times more sensitive to ethanol than D. magna at 24°C.

Table 6. Acute toxicity of ethanol to Daphnia magna and Ceriodaphnia dubia at 20°C and at 24°C

	48-h LC50 and 95% confidence interval (mgL^{-1})		
	20°C		24°C
	Daphnia magna		
Dates of Test		Dates of Tests	
		03-30-86	14221 (12813 - 15804)
06-11-84	13248 (11825 - 15009)	02-12-85	12820 (9941 - 15386)
06-11-84	11853 (10705 - 13294)	02-06-85	14136 (11046 - 15780)
05-21-84	11967 (10703 - 13599)	01-30-85	13470 (12310 - 14724)
		07-18-84	10860 (9721 - 12237)
		07-17-84	9268 (8408 - 10367)
geometric mean	12340 (11065 - 13948)		12318 (10596 - 13885)
		Ceriodaphnia dubia	
05-21-84	6386 (5364 - 7461)	06-04-84	5577 (4761 - 6557)
05-21-84	6772 (5955 - 7710)	06-04-84	3715 (3046 - 4432)
05-23-84	6325 (5383 - 7413)	06-04-84	6076 (5231 - 7115)
geometric mean	6492 (5561 - 7527)		5012 (4233 - 5913)

A point worthy of consideration is the difference in response to ethanol exhibited by these two organisms. Healthy *D. magna* neonates weigh around 18 µg dry (Cowgill et all 1986). Healthy *C. dubia* neonates weigh around 4 µg (Cowgill, unpublished data-dry weight). *C. reticulata* (Cummins et all 1969) of unspecified size class was estimated to weigh 6 µg. The latter is probably a young juvenile since the neonates of *C. dubia* and *C. reticulata* appear to be about the same size though the taxonomy is still in question (cf Berner 1986). This difference in response to ethanol exhibited by these two cladocerans is probably due to their vast difference in size. Although toxicity and body size are well known to be related in higher animals, there has been little discussion in this regard for aquatic species. Anderson and his co-workers (1973, 1975, 1980a, 1980b) have addressed this point in detail for fishes.

Although the present published standard practice recommended by the American Society of Testing and Materials (1980) is presently undergoing its required five year revision it should be realized that the requirement of <0.5 mL/L ethanol for D. magna will not create added problems in the execution of the static acute test but it may prove detrimental to C. dubia at either temperature.

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