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Toxicity of N,N-Dimethylformamide Used as a Solvent in Toxicity Tests with the Green Alga, *Selenastrum capricornutum*

J. S. Hughes[1] and A. G. Vilkas[2]

Union Carbide Corporation Environmental Services, Tarrytown, NY 10591

An investigator seeking to determine the toxicity of an insoluble material to aquatic organisms may choose to use a solvent to disperse the test material. Acetone, methanol, ethanol, triethylene glycol, and dimethylformamide are some suggested solvents (APHA et al. 1975). The highest recommended concentration of solvent in static systems is 0.5 mL/L. (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975). This value is based upon experience with fish and invertebrates. Information on the tolerance of algae to solvents, however, is scarce. STRATTON et al. (1980) investigated the effects of acetone on three species of Anabaena. Effects ranged from inhibition to stimulation, and varied with species. KLEPPEL & MCLAUGHLIN (1980) found that 0.1 mL/L acetone had no effect upon the growth of Skeletonema costatum.

The algal assay bottle test (AABT) (MILLER et al. 1978) is the most commonly used tool for assessing the effect of a potentially toxic substance on the growth of a selected species of algae. In this investigation, the AABT was used to assess the effects of N,N-dimethylformamide on Selenastrum capricornutum. S. capricornutum is a unicellular, non-motile, freshwater green alga which has been recommended by the U.S. EPA for use in the AABT (MILLER et al. 1978; EPA 1980). N,N-Dimethylformamide (DMF), because of its high ability to dissolve many organic compounds, low volatility, and low toxicity to aquatic animals, is an excellent solvent for aquatic studies.

Additionally, in comparison to a solvent such as acetone, DMF is less readily metabolized by bacteria. In a static system, such reduced metabolism results in less depletion of dissolved oxygen, an important consideration in toxicity tests involving fish and invertebrates. Dissolved oxygen is not of concern in algal toxicity tests; however, investigators may elect to use DMF in tests with algae as well as fish and invertebrates to maintain consistency among tests.

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[1]Current address: Malcolm Pirnie,Inc.,2 Corporate Park Dr., [2]Box 751, White Plains, New York 10602
[2]Current address: Union Carbide Corp., P.O. Box 444, Somerset, New Jersey 08873

METHODS AND MATERIALS

S. capricornutum used in this assay came from laboratory stock cultures. The original culture was obtained from the National Eutrophication Research Program, Pacific Northwest Environmental Research Laboratory, U.S. EPA, Corvallis, OR. Stock cultures were maintained in synthetic algal assay nutrient medium (MILLER et al. 1978) in continuously shaken (100 rpm) Erlenmeyer flasks under constant illumination of 4304±650 lumens/m^2 and temperature of 24±2C. A portion of stock culture was transferred into fresh medium to provide a week-old culture for assay inoculation.

All glassware used in testing was cleaned and sterilized as described by MILLER et al. (1978). Test vessels were 250-mL Erlenmeyer flasks fitted with foam stoppers to provide free gas exchange with the atmosphere. Medium used for testing was prepared as described by MILLER et al. (1978); sterilization was accomplished by filtration through a sterile 0.22-um porosity membrane filter.

Test concentrations were prepared by adding appropriate amounts of reagent grade DMF to nutrient medium in volumetric flasks to yield final concentrations of 0.10, 0.18, 0.32, 0.56 and 1.00 mL/L. After thorough mixing, 60 mL of each concentration was added to each of three replicate test vessels. The control contained medium only, 60 mL in each of three replicates. Test concentrations are expressed as ml/L rather than as mg/L since the assay was conducted to determine the amount of solvent carrier (usually measured as volume) that may be used.

An algal inoculum was prepared by centrifuging a portion of a seven-day-old stock culture, decanting the supernatant, and resuspending the cells in filter-sterilized $NaHCO_3$ (15 mL/L). The sample was centrifuged and resuspended once more before determining population density with a hemacytometer and microscope. The washed stock culture was diluted to a concentration of 19.33 x 10^4 cells/mL ($\bar{X}$,N=4). A 0.93 mL volume of this culture was aseptically added to each flask, yielding a nominal initial concentration of 3,000 cells/ml.

Flasks were kept in a Psycrotherm Controlled Environment Incubator Shaker at a temperature of 24±2C. Flasks were continuously shaken at 100 rpm, and continuous illumination of 4304±650 lumens/m^2 was provided by overhead cool-white fluorescent lights. Flasks were randomly repositioned each day to minimize spatial differences in the incubator.

Cell counts were made with an Improved Neubauer hemacytometer, 0.1 mm deep, on test days 0, 1, 2, 3, 4, 7, 9, 11 and 14. Four counts per replicate were made. All counts were multiplied by the appropriate conversion factor to yield cells/mL. Whenever feasible, 400 cells per replicate were counted in order to obtain ±10%

accuracy at the 95% confidence level. Samples were collected aseptically in disposable pipettes, and test flasks were swirled while pipetting to ensure representative sampling.

Growth of the test alga was expressed as maximum standing crop (MSC) in dry weight and in cell numbers. The maximum standing crop in any flask is defined as the maximum biomass achieved during incubation. For practical purposes, it may be assumed that the MSC is obtained within 14 days or whenever the increase in biomass is less than 5% per day. The assay was terminated when populations in the majority of the flasks had reached MSC. Dry weight was then determined using the filtration method of MILLER et al. (1978).

Mean maximum standing crop values, expressed both as cells/mL and as mg/L, were tested for homoscedasticity (equality of variances) using the variance ratio test (STEEL & TORRIE 1980). One-way analysis of variance (ANOVA) (STEEL & TORRIE 1980) was performed on homoscedastic data. DUNCAN'S (1955) new multiple range test was used to locate significant differences among treatment means, where applicable. All tests of significance are at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Cell counts during the 14 day exposure of S. capricornutum to DMF are presented in Table 1. These data are plotted in Figure 1. Each curve represents the mean of three replicates. The value for each replicate in turn is the mean of four sample counts.

Maximum standing crop data, (MSC), expressed as cells/mL, are presented in Table 2. MSC occurred by day 7 or 9 in all test concentrations and the control. ANOVA and DUNCAN'S test indicated that none of the mean MSC (cells/mL) values were significantly different from that in the control.

Dry weight in mg/L (Table 2) may also be considered as an expression of maximum standing crop. ANOVA and DUNCAN'S test indicated that the mean dry weight in the highest test concentration (1.0 mL/L) was significantly greater than that in the control. There were no significant differences between the mean dry weights in any other concentrations and the control.

The no observed effect concentration (NOEC) is defined as the highest concentration tested that had no significant effect upon maximum standing crop (cells/mL or dry weight,mg/L). Since 1.0 mL/L caused a significant increase in dry weight, the NOEC is 0.5 mL/L.

As determined by the algal assay bottle test, the no observed effect concentration (NOEC) for S. capricornutum exposed to DMF is 0.5 mL/L. This suggests that as much as 0.5 mL/L DMF may be used as a solvent in the bottle test. A solvent control, comprised of

Table 1. Cell counts (cells/mL x 10^4) during 14-day exposure of S. capricornutum to N,N-dimethylformamide

Nominal concentration mL/L		Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 9	Day 11	Day 14
0	A	0.25	0.28	1.53	4.78	19.0	310.5	402.0	375.0	388.0
	B	0.28	0.31	2.03	5.08	20.0	309.0	373.0	381.0	310.0
	C	0.22	0.36	1.19	5.47	15.7	314.0	356.0	332.0	309.0
	Mean	0.25	0.32	1.58	5.11	18.2	311.2	377.0	362.7	335.7
	SD[1]	0.03	0.04	0.42	0.35	2.2	2.6	23.3	26.7	45.3
0.10	A	0.28	0.25	1.53	5.22	26.1	403.0	392.0	420.0	424.0
	B	0.22	0.30	1.67	9.06	29.5	423.0	436.0	400.0	410.0
	C	0.25	0.42	1.72	6.25	23.3	355.0	425.0	399.0	342.0
	Mean	0.25	0.32	1.64	6.84	26.3	393.7	417.7	406.3	392.0
	SD	0.03	0.09	0.10	1.99	3.1	35.0	22.9	11.8	43.9
0.18	A	0.22	0.33	1.72	5.86	23.6	344.0	407.0	445.0	394.0
	B	0.33	0.28	1.72	6.19	22.9	397.0	423.0	388.0	384.0
	C	0.17	0.31	1.56	5.33	22.8	404.0	394.0	453.0	380.0
	Mean	0.24	0.31	1.67	5.79	23.1	381.7	408.0	428.7	386.0
	SD	0.08	0.03	0.09	0.43	0.4	32.8	14.5	35.4	7.2
0.32	A	0.19	0.31	1.95	7.06	31.2	278.0	433.0	397.0	385.0
	B	0.33	0.45	1.75	6.69	29.2	428.0	463.0	433.0	379.0
	C	0.19	0.31	2.17	7.22	26.4	388.0	424.0	413.0	421.0
	Mean	0.24	0.36	1.96	6.99	28.9	364.7	440.0	414.3	395.0
	SD	0.08	0.08	0.21	0.27	2.4	77.7	20.4	18.0	22.7
0.56	A	0.08	0.28	1.58	6.28	24.8	349.0	396.0	417.0	360.0
	B	0.22	0.14	1.75	6.47	23.9	365.0	459.0	381.0	399.0
	C	0.17	0.14	2.03	6.58	21.7	386.0	415.0	378.0	417.0
	Mean	0.16	0.19	1.79	6.44	23.5	366.7	423.3	392.0	392.0
	SD	0.07	0.08	0.23	0.15	1.6	18.6	32.3	21.7	29.1
1.00	A	0.17	0.36	2.16	6.53	24.4	355.0	389.0	374.0	397.0
	B	0.14	0.39	1.53	5.81	27.1	364.0	395.0	371.0	317.0
	C	0.14	0.53	2.06	5.56	26.9	345.0	360.0	393.0	376.0
	Mean	0.15	0.43	1.92	5.97	26.1	354.7	381.3	379.3	363.3
	SD	0.02	0.09	0.34	0.50	1.5	9.5	18.7	11.9	41.5

[1] SD = standard deviation

Table 2. Maximum staging crop expressed as cells/mL ($\times 10^4$) and as dry weight, mg/L

Nominal Concentration mL/L	MSC, cells/mL cells/mL	MSC, cells/mL Day attained	MSC, dry weight mg/L
0 A	402.0	9	126.2
B	373.0	9	132.7
C	356.0	9	124.9
Mean	377.0		127.9
SD[1]	23.3		4.2
0.10 A	403.0	7	129.9
B	423.0	7	134.8
C	425.0	9	132.6
Mean	417.0		132.4
SD	12.2		2.4
0.18 A	407.0	9	143.2
B	397.0	7	133.7
C	404.0	7	133.7
Mean	402.7		136.9
SD	5.1		5.5
0.32 A	433.0	9	133.6
B	428.0	7	121.2
C	388.0	7	140.2
Mean	416.3		131.7
SD	24.7		9.6
0.56 A	396.0	9	146.0
B	459.0	9	130.9
C	386.0	7	136.9
Mean	413.7		137.9
SD	39.6		7.6
1.00 A	355.0	7	145.3
B	364.0	7	157.3
C	345.0	7	145.6
Mean	354.7		149.4
SD	9.5		6.8

[1] SD = Standard deviation

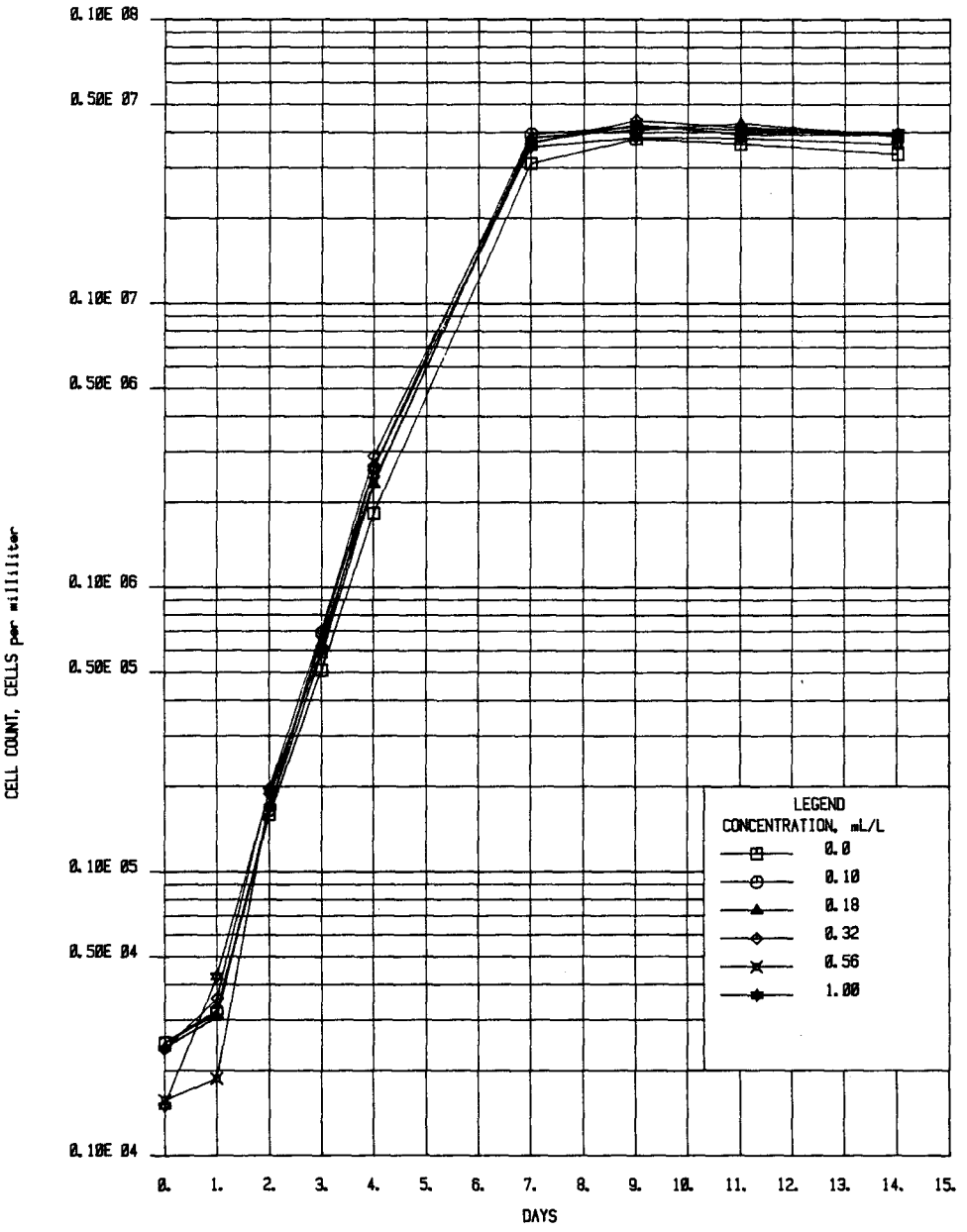

Figure 1. Mean Cell Counts Versus Time for 14-Day Exposure of Selenastrum capricornutum to N,N-Dimethylformamide

replicate flasks containing an amount of solvent equivalent to the greatest amount of solvent used in any test concentration, should be included in any assay employing a solvent. Growth in the test concentrations should be expressed relative to that in the solvent control, and any significant differences between growth in the control and solvent control should be determined.

Further testing is necessary to determine the concentration of DMF that is toxic to S. capricornutum. Additional studies should be conducted to ascertain the responses of other species of algae to DMF.

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