20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish

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Resource Publication 37 • Washington, D.C. • April 1967

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ABSTRACT.--Toxicities of dimethyl sulfoxide (DMSO) to rainbow trout, brook trout, lake trout, carp, black bullhead, channel catfish, green sunfish, bluegill, and yellow perch were determined in 24-, 48-, and 96-hour static bioassays at 12° C. Toxicity was of low order, around 30 p.p.t. Water quality had little effect, but increased temperature increased the toxicity to rainbow trout. A preliminary test indicated that DMSO has little effect on the toxicity of antimycin to bluegill.

Dimethyl sulfoxide (DMSO) is the simplest of the homologous series of organic sulfoxides; it is prepared by oxidation of dimethyl sulfide. Its formula is $(CH_3)_2SO$, and the pure form is a hygroscopic, colorless, odorless liquid, melting at 18.45° and boiling at 189° C., with a specific gravity of 1.100 (Stecher, 1960).

DMSO was first synthesized in 1867 but remained a laboratory curiosity until the 1940's when it was successfully used as a solvent for the spinning of polyacrylonitrile fibers (Block, 1964). The first physiochemical data on the compound appeared in 1948, and since that time DMSO has been used extensively in several fields.

DMSO has been shown to possess remarkable potential as a solvent for many types of inorganic and organic compounds including gases (Willson et al., 1965). It has also been used as a preservative during freeze storage of red blood cells (Lovelock and Bishop, 1959), platelets (Geisler et al., 1964), spermatozoa (Sherman, 1964), mitochondria (Greiff, 1961), protozoa (Hwang, 1964), bone marrow (Ashwood-Smith, 1961a; Persidsky and Richards, 1963), cardiac muscle (Levy et al., 1962), and tissue culture cells (Dougherty, 1962; Porterfield and Ashwood-Smith, 1962). DMSO also exhibited radioprotective action against lethal doses of X-irradiation in mice (Ashwood-Smith, 1961b).

In the medical field, the compound is under investigation as a penetrant carrier, local analgesic, anti-inflammatory adjunct, bacteriostatic agent, diuretic, tranquilizer, and potentiator (Jacob et al., 1964a). It is even reported to be good for headaches (Jacob, 1965). The most promising of these areas appears to be the ability of DMSO to penetrate biological membranes and act as a carrier for other drugs (Horita and Weber, 1964; Jacob et al., 1964b; Stoughton and Fritsch, 1964; Stoughton, 1965).

In the agricultural field, DMSO has been shown to be of value as a solvent carrier for certain compounds used in the control of plant diseases (Bean, 1965; Keil et al., 1965). It also has some herbicidal activity when used by itself for the control of purple nutsedge (Anderson and Dunford, 1966).

Additional information and references on DMSO can be obtained from articles written by Rosenkrantz et al. (1963), and Kligman (1965a and 1965b).

These interesting properties of DMSO plus the fact that it is miscible in all proportions with water suggested that it might be useful

in fisheries as a nontoxic solvent in toxicity studies and for the administration of non-water-soluble drugs to fish. In addition, if DMSO enhances the absorption by fish of compounds dissolved in it, a major breakthrough in the fields of fish control and fish disease control would result. It is for these purposes that the following toxicity studies were undertaken.

MATERIALS AND METHODS

Samples of DMSO were obtained from Ayerst Laboratories, New York, N.Y. The formulation was 90 percent DMSO and 10 percent water.

The fish were obtained from seveial fish hatcheries (table 1), and were introduced to the static bioassays after routine acclimation as described by Lennon and Walker (1964).

Preliminary bioassays were conducted in 1-gallon glass jars each containing 3 liters of bioassay media and two fish. After the approximate level of toxicity had been established, delineative bioassays were conducted in 5-gallon glass jars each containing 15 liters of reconstituted deionized water and 10 fish. The proper volume of bioassay media was maintained by removal of a quantity of water equal to the aliquot of DMSO which was to be added. Each test included 5 to 9 concentrations of chemical and 50 to 90 test fish plus 10 fish for controls.

Various water qualities were obtained by adding selected concentrations of reconstituting salts to deionized water (table 2). Tests were maintained at 7^{O}, 12^{O}, or 17^{O} C. by water baths.

Survival and mortality were recorded at 24, 48, and 96 hours. The data were analyzed by plotting concentration versus mortality on logarithmic normal (probability) graph paper to define the concentration which produced 50-percent mortality (LC_{50}), slope function, variation, and 95-percent confidence intervals (C.I.) as described by Litchfield and Wilcoxon (1949).

All results are reported in parts per thousand (p.p.t.), by volume, of total material added to the test vessel instead of active ingredient.

TABLE 1.--Species, sizes, and sources of bioassay fish

Species	Average length (inches)	Average weight (grams)	Source
Rainbow trout, Salmo gairdneri	1.8	0.	NFH, Manchester, Iowa.
Do	1.8	1.	Rainbow Ranches, Spokane, Wash.
Brook trout, Salvelinus fontinales	1.5	0	SFH, Osceola, Wis.
Lake trout, Salvelinus namaycush	1.9	0	SFH, St. Croix Falls, Wis.
Carp, Cyprinus carpio	1.6	1.	NFH, Take Mills, Wis.
Black bullhead, Ictalurus melas	2.0	1.	NFH, New London, Minn.
Channel catfish, Ictalurus punctatus	2.0	1.	NFH, Fairport, Iowa.
Green sunfish, Lepomis cyanellus	1.5	0.	NFH, Lake Mills, Wis.
Bluegill, Lepomis macrochirus	1.3	0.	Do.
Yellow perch, Perca flavascens	2.6	1.	Do.

[1] NFH = National Fish Hatchery; SFH = State Fish Hatchery.

TABLE 2.--Composition and analysis of reconstituted, deionized water used in bioassays

Classification of water	Amount of salts added (mg./l.)				pH range	Range of total hardness as p.p.m. $CaCO_3$	Range of total alkalinity as p.p.m. $CaCO_3$
	NaHCO3	$CaSO_4$	$MgSO_4$	KCL			
Soft	12.0	7.5	7.5	0.5	6.4 - 6.8	10-13	10-13
Medium	48.0	30.0	30.0	2.0	7.2 - 7.6	40-48	30-35
Hard	192.0	120.0	120.0	8.0	7.6 - 8.0	160-180	110-120

[1] Standard reconstituted water used in routine bioassays.

RESULTS AND DISCUSSION

PRELIMINARY TESTING

Preliminary tests to determine the approximate level of DMSO toxicity were carried out with yellow perch as the test species. In order to preserve the supply of test material it was necessary to prepare a 3-liter solution containing 500 p.p.t. of DMSO. Subsequent concentrations were obtained by dilution of this solution.

Results in terms of the approximate time to death at each concentration were as follows:

500 p.p.t. - 10 minutes
250 p.p.t. - 30 minutes
125 p.p.t. - 1.5 hours
62 p.p.t. - 24 hours
31 p.p.t. - no mortality within 96 hours

From these data, the 24-, 48-, and 96-hour LC_{50} values appear to lie between 30 and 60 p.p.t., and concentrations were selected accordingly for the routine toxicity bioassays.

GENERAL TOXICITY

DMSO exhibited a consistent and nonselective toxicity of very low order to the nine species tested (table 3).

Probit analysis yielded slope functions on logarithmic paper which ranged from 1.06 to 1.20 with a mean of 1.11 on all tests. This indicates that as the level of acute toxicity is approached a minimal change in concentration is required to produce either 0- or 100-percent mortality.

The comparative resistance of the nine species was extremely close. The 96-hour LC_{50}'s ranged from only 32.3 to 43.0 p.p.t. for the various species.

The order of susceptibility of the nine species varied with the observation period. At 24 and 48 hours, for example, channel catfish were the most susceptible, with bluegill the most resistant at 24 hours and yellow perch the most resistant at 48 hours. By 96 hours, rainbow trout became the most susceptible and green sunfish the most resistant. This fluctuation in the comparative order of sensitivity further exemplifies the extremely nonselective toxicity to all fish tested.

Recent studies at the Western Fish Nutrition Laboratory with yearling coho salmon (Oncorhynchus kisutch) determined the median tolerance limit (TLm) to be 72, 55, and 46 p.p.t. at 24. 48, and 96 hours respectively.[1] Ball (1966) reported that the 48-hour LC_{50} of DMSO to goldfish (Carassius auratus) is 43 p.p.t. at 15 C. These results are in close agreement with the results at this laboratory and serve to further substantiate the consistent and nonselective toxicity of DMSO.

[1] Personal communication from Pete Benville, Jr., Chemist, Western Fish Nutrition Laboratory, Bureau of Sport Fisheries and Wildlife, Cook, Wash., 1966.

TABLE 3.--Toxicity of 90-percent DMSO to nine species of fish at 12° C.

Species	At 24 hours		At 48 hours		At 96 hours	
	LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.	LC50 (p.p.t.)	95-percent C.I.
Rainbow trout	53.0	48.6 - 57.8	41.7	39.3 - 44.2	32.3	30.2 - 34.6
Brook trout	54.5	50.9 - 58.3	46.0	42.2 - 50.1	36.5	33.2 - 40.2
Lake trout	47.8	42.3 - 54.0	38.2	35.4 - 41.3	37.3	35.2 - 39.5
Carp	44.0	39.3 - 49.3	44.0	39.3 - 49.3	41.7	36.3 - 48.0
Black bullhead	42.5	37.9 - 47.6	39.2	35.3 - 43.5	36.5	33.8 - 39.4
Channel catfish	39.0	36.1 - 42.1	34.5	31.7 - 37.6	32.5	29.8 - 35.4
Green sunfish	65.0	61.3 - 68.9	52.5	47.7 - 57.8	43.0	35.8 - 51.6
Bluegill	72.0	63.2 - 82.1	56.0	51.9 - 60.5	33.5	29.9 - 37.5
Yellow perch	65.0	61.3 - 68.9	57.0	52.3 - 62.1	37.0	33.9 - 40.3

Rabinowitz and Myerson (1966) stated that a concentration of 19 p.p.t. of DMSO produced an approximate 48-hour LD_{50} with neon tetras (Paracheirodon innesi), platys (Xiphophorus maculatus), mollies (Pescilia latipinna), and guppies (Poecilia reticulata). The LD_{50}'s for zebras (Brachydanio rerio) and catfish (Corydoras paleatus) were somewhere in excess of 25 p.p.t. These results were obtained in distilled water at 24° to 25° C. and the increased toxicity indicated may be the result of osmotic stress induced by the test media.

EFFECT OF WATER QUALITY AND TEMPERATURE ON TOXICITY

Changes in water quality at 12° C. had little or no effect upon the toxicity of DMSO (table 4). The LC_{50} confidence interval in any particular water quality overlaps the LC_{50}'s in other water qualities within the same observation period in all cases except one. This exception was in hard water at 96 hours. In general, it appears that DMSO is slightly less toxic in hard water than it is in waters of soft or medium hardness.

Changes in temperature at medium hardness exhibited a substantial influence on toxicity (table 4). An increase in toxicity in excess of 10 p.p.t. as the temperature increases from 7° to 17° C. was observed at all observation periods.

This increase in toxicity at warmer temperature is in agreement with observations made at the Western Fish Nutrition Laboratory.

EFFECT OF DMSO ON TOXICITY OF ANTIMYCIN

A preliminary test was performed to determine what, if any, influence DMSO has on the toxicity of antimycin to bluegill. Various concentrations of antimycin were added in combination with enough DMSO to produce 1.0 p.p.t. of DMSO in the test vessel. A comparison test was run using only acetone as solvent for the antimycin.

The 96-hour LC_{50} of antimycin and acetone alone was 0.089 parts per billion (p.p.b.), while antimycin in combination with 1.0 p.p.t. of DMSO produced a 96-hour LC_{50} of 0.084 p.p.b. These results reflect biological variation and indicate that DMSO has no effect on the toxicity of antimycin at 96 hours. It is possible that in a bioassay designed to yield toxicity with shorter exposures, DMSO could enhance the absorption of antimycin sufficiently to affect toxicity.

Ball (1966) compared the relative toxicity of 0.05 p.p.m. p,p'-DDT to goldfish when used in combination with 6 and 18 p.p.t. of either DMSO or acetone. His results indicated that DMSO does not significantly affect the median survival time of goldfish when compared to acetone. He further suggested that DMSO may be a better solvent than acetone for pesticide toxicity studies.

Rabinowitz and Myerson (1966) were unable to show a significant difference in the uptake by aquarium fish of radioactive labeled dyes when used in combination with 1.0 p.p.t. of DMSO.

TABLE 4.--Effect of water quality and temperature on toxicity of DMSO (90-percent) to rainbow trout

Temperature C.	Water quality	At 24 hours		At 48 hours		At 96 hours	
		LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C. I.
7°	medium	65.5	57.0-75.3	46.0	41.8-50.6	41.5	37.7-45.6
12°	medium	53.0	48.6-57.8	41.7	39.3-44.2	32.3	30.2-34.6
17°	medium	41.5	37.7-45.6	35.0	31.8-38.5	27.7	25.0-30.7
12°	soft	53.5	49.1-58.3	42.3	38.4-46.5	33.5	30.7-36.5
12°	hard	57.0	51.8-62.7	44.8	41.5-48.4	38.0	35.8-40.3

All of these results indicate that DMSO, when used as a diluted constituent, does not affect the absorption of some chemicals by fish. It has been shown to be an excellent solvent, and is worthy of further investigation as such for certain chemicals used in fisheries. In addition, the potential of DMSO as a penetrant carrier of certain drugs used in human medicine suggests investigation of similar potential in the treatment of fish disease.

CONCLUSIONS

1. The acute toxicity of DMSO to fish is of a very low order.

2. When the level of acute toxicity is reached, DMSO is abruptly and nonselectively toxic to the nine species tested.

3. Various water qualities at 12 C. have little effect upon the toxicity of DMSO to rainbow trout.

4. Increases in temperature cause a definite increase in the toxicity of DMSO to rainbow trout.

5. Preliminary results indicate that 1.0 p.p.t. of DMSO has no effect on the toxicity of antimycin to bluegill at 96 hours.

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