Dissolved Oxygen Depletion in Static Bioassay Systems

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Acute toxicity tests employing static bioassays are generally conducted for 24, 48 or 96 hours. There is concern over the frequency with which dead test organisms should be removed from the system and whether or not artificial aeration should be allowed. Understanding bioassay oxygen regimes is important because of the relationship between dissolved oxygen concentration (D.O.) and toxicity of many substances. The mechanism responsible for toxicity in the presence of reduced oxygen concentration should be considered (SOUTHGATE et al. 1933; WUHRMANN 1952; WUHRMANN and WOKER 1953; DOWNING 1954; WEISS and BOTTS 1957; ALLERDICE and BRETT 1957; CAIRNS and SCHEIER 1958; LLOYD 1961; SAUNDERS 1962; HERBERT and SHURBEN 1963; BURDICK 1967; PICKERING 1968; BROWN 1968; SPRAGUE 1969, 1970, 1971, 1973; HOKANSON and SMITH 1971; LOCH and MACLEOD 1974). It appears that the greater toxicity of substances at lower D.O. concentrations is due to changes in physiological reactions by test fish such as increased respiration and mass flow over gills.

Even if dead organisms were removed as soon as detected, there could still be a significantly long period of time, up to 15 hours during the night, when dead test fish would not be observed. We investigated the possibility that dead animals, as possibly encountered in bioassays after the laboratory staff had gone home for the evening, could impose a detrimental B.O.D. on the system.

Bioassay jars (3.9 ℓ) filled to a depth of 18 cm were set up according to AMERICAN PUBLIC HEALTH ASSOCIATION (1971) specifications and not more than approximately 1 g of fish/ℓ was employed. Bluegill (Lepomis macrochirus Rafinesque) averaging 0.75 g were used as test organisms and were allowed to acclimate to laboratory conditions in an aerated flow-through tank. During tests the fish were handled as little as possible; a small dip net was employed to avoid agitation of the water's surface and consequent oxygenation. Water from the acclimation tank was used in the tests and was allowed to stand in the jars 48 hours prior to introduction of fish. D.O. was monitored at 4 hour intervals over each 24 hour test period by a Y.S.I. Model 51 oxygen meter and also by the azide modification of the Winkler method at the termination of each test.

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Tests were conducted considering the number of dead fish, live fish or temperature as variables. Four test jars in duplicate at 25 C and 4 test jars in duplicate at 36 C were started at air saturation. At the start of the test (time 0) 0, 1, 3 or 5 freshly euthanized fish, respectively, were placed into each jar which was then monitored for D.O. change. The last test consisted of 4 test jars in duplicate at 25 C with 5 live fish/jar. Following 48 hours acclimation in the jars, some fish were randomly captured, euthanized and immediately returned to their respective jars (time 0) at the following rates: 0 dead and 5 live fish, 1 dead and 4 live fish, 3 dead and 2 live fish, and 5 dead fish. Data obtained from replicate jars were virtually identical. Hence, means of the D.O. of the duplicate treatments are presented.

Results indicate that there is an inverse relationship between dead fish biomass and D.O. in the system (Table 1).

TABLE 1

Average Dissolved Oxygen Concentrations (ppm) in Static Bioassays for a 24 Hour Period following Introduction of Dead Fish at Time 0. Ambient Temperature = 25 C.

Sampling Time (Hour)	No. Dead Fish g Dead Fish	0 0	1 0.65	3 2.32	5 3.34
0		8.5	8.6	8.7	8.9
4		8.5	8.2	8.2	8.3
8		8.1	7.7	7.5	7.6
12		8.0	7.5	6.9	6.9
16		8.0	7.1	5.8	5.0
20		8.0	6.6	4.1	1.6
24		8.0	5.7	1.5	0.2

Similarly, the greater the amount of decomposing tissue the more rapid the decline in oxygen from saturation levels. At 36 C the initial D.O. was lower than at 25 C due to decreased saturation with elevated temperature. Even one dead individual at the higher temperature caused a depletion of oxygen to levels generally unacceptable for survival of fish (Table 2). Comparisons of Tables 1 and 3 indicate that 5 live individuals

TABLE 2

Average Oxygen Concentrations (ppm) in Static Bioassays for a 24 Hour Period following Introduction of Dead Fish at Time 0. Ambient Temperature = 36 C.

Sampling Time (Hour)	No. Dead Fish g Dead Fish	0 0	1 0.8	3 2.1	5 3.5
0		5.7	5.9	5.8	5.9
4		5.6	5.3	4.9	5.0
8		5.5	3.8	1.1	0.1
16		5.7	0.2	<0.1	0.0
20		5.8	0.1	<0.1	0.0
24		5.8	0.5	<0.1	0.0

can lower the D.O. to a level 75% below air saturation at 25 C. In systems with both live and dead fish (Table 3) there was

TABLE 3

Average Dissolved Oxygen Concentrations (ppm) in Static Bioassays for a 24 Hour Period following Sacrifice of Live Fish (Time 0) in Systems at Equilibrium Dissolved Oxygen Levels. Ambient Temperature = 25 C. * Indicates fish breathing at surface.

Sampling Time (Hour)	No. Fish g Live g Dead	5 Live 3.4 0	4 Live/1 Dead 2.3 0.4	2 Live/3 Dead 1.4 2.2	5 Dead 0 3.4
0		2.2	2.7	2.4	2.5
4		2.3	2.9	2.5	3.3
8		2.2	2.7	2.4	3.4
12		2.3	2.6	1.9	3.1
16		2.2	2.1	0.8	1.2
20		2.1	1.3	0.1*	0.1
24		1.8	0.7*	<0.1*	<0.1

initial stabilization or slight increase in D.O. due to lack of respiration by the euthanized fish. Thereafter, the D.O. declined rapidly. A combination of 2 live and 3 dead fish at 25 C resulted in "overnight" oxygen depletions to levels below 1 ppm and below minimum sustainable concentrations necessary for health of fish.

The above observations suggest that biomass/water volume differentials employed in such bioassay systems is not sufficient to ensure against serious depletion of oxygen by dead test organisms. It is probable, therefore, that dead individuals in an aquatic bioassay may influence toxicity of test substances to the remaining living fish within the system.

References

ALLERDICE, D. F., and J. R. BRETT: J. Fish. Res. Bd. Can. 14, 783 (1957).

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard methods for the examination of water and wastewater. 13 ed. New York (1971).

BROWN, V. M.: Water Res. 2, 723 (1968).

BURDICK, G. E.: Am. Fish. Soc., Spec. Pub. 4, 7 (1967).

CAIRNS, J., and A. SCHEIER: Proc. 12 Indust. Waste Conf., Purdue Univ. Engin. Ext. Ser. 94, 165 (1958).

DOWNING, K. M.: J. Exp. Biol. 31, 161 (1954).

HERBERT, D. W. M., and D. S. SHURBEN: Ann. Appl. Biol. 52, 321 (1963).

HOKANSON, K. E. F., and L. L. SMITH, JR.: Trans. Amer. Fish. Soc. 100, 1 (1971).

LLOYD, R.: J. Exp. Biol. 38, 447 (1961).

LOCH, J. S., and J. C. MACLEOD: Can. Dep. Environ., Fish Marine Serv., Fish. Operat. Direct., Cent. Reg., Tech. Rept. Ser. CEN/T-74-2:31 p.

PICKERING, Q. H.: Water Res. 2, 187 (1968).

SAUNDERS, R. L.: Can. J. Zool. 40, 817 (1962).

SOUTHGATE, B. A., F. T. K. PENTELOW, and R. BASSINDALE: Biochem. J. 27, 983 (1933).

SPRAGUE, J. B.: Water Res. 3, 793 (1969).

SPRAGUE, J. B.: Water Res. 4, 3 (1970).

SPRAGUE, J. B.: Water Res. 5, 245 (1971).

SPRAGUE, J. B.: Biological methods for the assessment of water quality, Am. Soc. Test. Materials, Spec. Tech. Pub. 528, 6 (1973).

WEISS, C. M., and J. L. BOTTS: Sewage Indust. Wastes 29, 810 (1957).

WUHRMANN, K.: Centre belge d'étude et de Document. des Eaux 15, 1 (1952).

WUHRMANN, K., and H. WOKER. Schweiz. Zeitschr. Hydrol. 15, 235 (1953).

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