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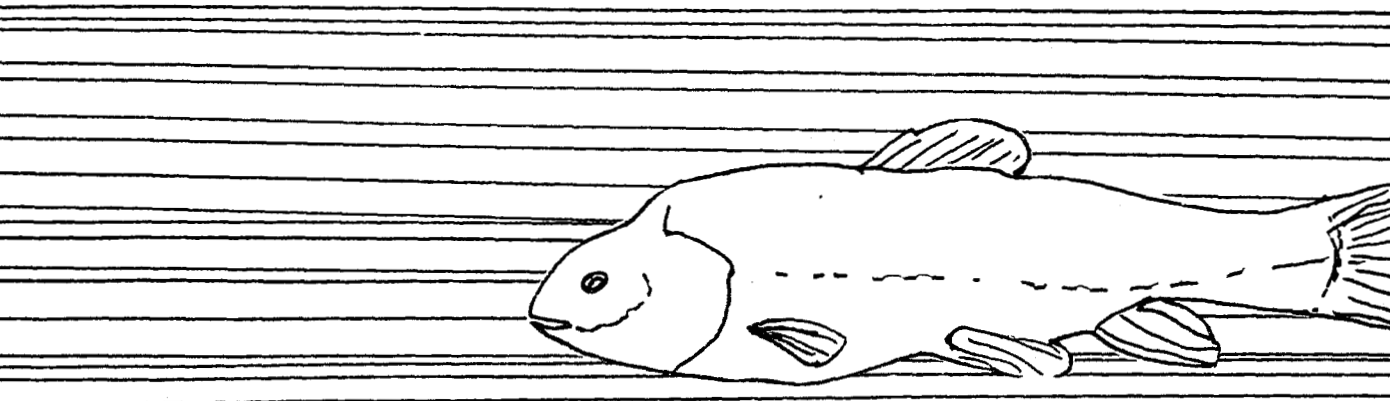
ACUTE TOXICITY OF 46 SELECTED DYES TO THE FATHEAD MINNOW, *Pimephales promelas*



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ACUTE TOXICITY OF 46 SELECTED DYES TO THE FATHEAD MINNOW, *Pimephales promelas*

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for

Ecology Committee
AMERICAN DYE MANUFACTURERS INSTITUTE, INC.

September, 1972

ABSTRACT

Forty-six commercially-important dyes were tested by bioassays, employing dye concentrations up to 180 mg/ℓ; 96 hr TL_{50} was determined. Static bioassays were conducted with fathead minnows (*Pimephales promelas*). The TL_{50} for 29 dyes was in excess of 180 mg/ℓ; for 3 dyes, between 100-180 mg/ℓ; for 14 dyes, at less than 100 mg/ℓ. The most toxic dyes were Basic Violet 1 (methyl violet), 0.047 mg/ℓ; Basic Green 4 (malachite green), 0.12 mg/ℓ; Disperse Blue 3, 1 mg/ℓ; Basic Yellow 11, 3.2 mg/ℓ; Basic Blue 3, 4 mg/ℓ; Acid Blue 113, 4 mg/ℓ; Basic Brown 4, 5.6 mg/ℓ; Mordant Black 11, 6 mg/ℓ; Acid Green 25, 6.2 mg/ℓ; and Acid Black 52, 7 mg/ℓ.

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INTRODUCTION

Man's use of dyes dates back to the Stone Age or earlier, and by 3000 B.C. the dyeing industry was well developed in Egypt, Mesopotamia, and India. The first synthesis of an organic dye was achieved in 1771 and since that time hundreds of new ones have been added to the dyer's repertoire.

Despite the long history of dyes and dyeing, little is known of the effects of dyes on living organisms. In 1970, the American Dye Manufacturer's Institute, Inc. initiated a number of research projects to evaluate the toxicity of dyes on selected organisms representative of receiving stream biota. In June, 1971, the UNC Wastewater Research Center began a study of the toxicity to fish and algae of 46 widely used dyes. This report presents results of acute toxicity bioassay studies with the fathead minnow, Pimephales promelas.

MATERIALS AND METHODS

The testing procedure was designed to adhere closely to the static bioassay described in thirteenth edition of Standard Methods for the Examination of Water and Wastewater (APHA et al. 1971). That procedure permits considerable flexibility and specific materials and methods employed in these tests are presented below.

The static bioassay method to evaluate toxicity has been criticized because it does not take into account many aspects of this question and more complex alternate methods have been suggested to overcome some of the difficulties (Standard Methods, APHA et al. 1971). Nevertheless, this type of bioassay has been, and will probably continue to be, widely used because of its relative simplicity and economy.

A major objection to reported fish bioassay results in general is that seldom has sufficient information been published on the test organism, dilution water and test conditions (Cairns, 1969; Kemp, Abrams, and Overbeck, 1971). A special effort has been made to avoid this criticism by preparing a data sheet designed to include all pertinent information for each test. The data sheet for each dye tested is reproduced in the Appendix.

Test Fish

The fathead minnow, Pimephales promelas, was selected from a list of recommended species prepared by Dr. D. I. Mount of the National Water Quality Laboratory (reported in Cairns, 1969). This species has been widely used in fish bioassay studies, proved adaptable to laboratory conditions, and was readily available locally. Test fish were

obtained from Berry Water Gardens and from Windmill Fish Hatcheries, both located in Kernersville, North Carolina.

New shipments of fish were routinely exposed on arrival to the broad-spectrum antibiotic tetracycline HCl (Tetrachel^{R}, Rachelle Laboratories, Inc., Long Beach, California) at a dose of 50 mg per gallon of water for 24-48 hours. Such treatment is necessary to prevent introduction into the stock tank of diseases from fishery stock or from fish damaged in shipment (Innes, 1966). Aureomycin (chlortetracycline) and related compounds are now commonly used for treatment (Innes, 1966). Prior to development of antibiotics brief exposure to bactercidal and fungicidal dyes, including malachite green, was practiced (Lewis, 1963). This use of malachite green is further described on page 14.

Upon any evidence of disease in the stock tanks, the tetracycline treatment was repeated.

Fish used in full-scale bioassay tests were maintained in a 250-gallon polyethylene tank equipped with aeration devices. Water was renewed continuously by introduction of fresh tap water from the City of Chapel Hill, pretreated to remove chlorine and organic carbon (see below). A thermostatic device prevented the temperature from dropping below 14^{o} C.

Fish were acclimatized to test temperature and the experimental dilution water for a minimum of ten days before testing. They were fed 5-7 times per week with protein-enriched commercial fish food. For 48 hours prior to testing, the fish were starved, as customary in static tests to reduce the amount of waste materials generated by them in

Figure 1
Fathead Minnows

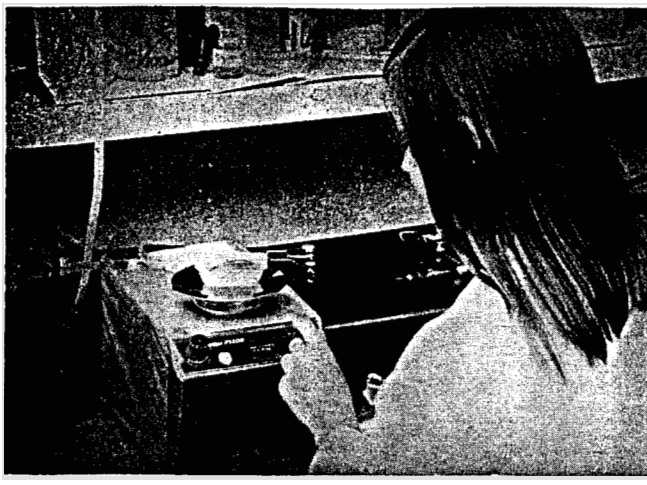


Figure 2
Weighing Fish

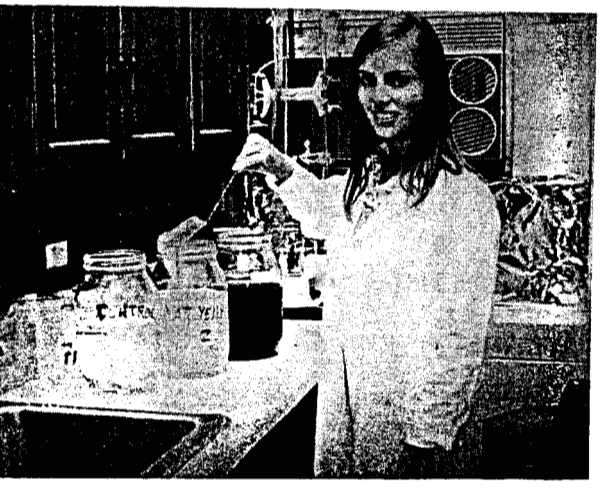


Figure 3
Preparation for
Preliminary Bioassay Studies

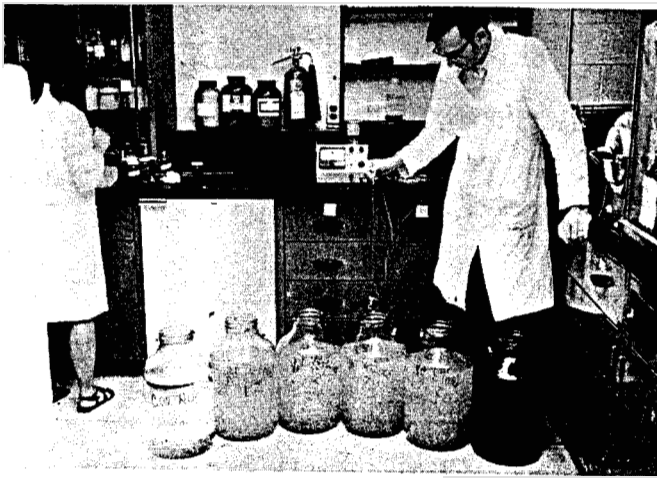


Figure 4
Temperature and Dissolved Oxygen
Determinations in Full-Scale
Bioassay Studies

the test container. The wastes would stimulate bacterial growth and lead to increased oxygen consumption and, in addition, might more directly affect test results by reacting chemically with the material being tested.

Experimental Dilution Water

The purpose of the bioassays was not to evaluate the effect of dye waste discharges on fish native to some specific receiving stream, but, instead, to evaluate the effects of a large number of dyes on one fish species. Since reproducibility and general application of the results were desired, experimental dilution water of constant quality was essential. To obtain such water, Chapel Hill tap water was dechlorinated and filtered by passage through an activated carbon--sand filter system (Carbo-Dur and coarse sand system, Permutit Water Conditioning, Inc.). The water was analyzed according to Standard Methods (APHA et al. 1971) before each test series, as shown in the Appendix. Its average chemical and physical characteristics are shown in Table I.

TABLE I. CHARACTERISTICS OF EXPERIMENTAL DILUTION WATER

Parameter	Range	Median
pH	6.6-7.1	6.8
Total organic carbon, mg/l	2-34	4
Fe, mg/l	<0.01-0.63	<0.02
Al, mg/l	<1.0	<1.0
Mg, mg/l	0.53-1.6	1.4
Ca, mg/l	5.1-8.7	8.5
Total dissolved solids, mg/l	85-138	121
Turbidity, JTU	0-2	0
Total alkalinity as $CaCO_3$, mg/l	19-32	27

Dyes

Dyes to be tested were supplied by ADMI in June and July, 1971. The 46 dyes represented composites from a number of manufacturers (see Appendix). Table II presents the color index number and name, and common names for each of the 46 dyes (Lillie, 1969; Salle, 1967; Society of Dyers and Colorists, 1956-58). Current commercial names are available from the annual AATCC publication Products/73 or from the Color Index.

Dyes supplied as liquids were initially stored at ca. 20° C and subsequently refrigerated. Dry powders were stored in the dark at ca. 20° C. All were stored in the containers in which they were received.

Concentrations of dyes are expressed in terms of milligrams per liter (mg/l) of the dye. For dyes supplied as dry powders, the weight is that of the powder; no attempt was made to determine the concentration of trace and inert materials. For dyes supplied as liquid solutions or suspensions, the weight of the dye was calculated from information supplied by ADMI. For example, for a 15% (by weight) dispersion of Disperse Yellow in 15% Reax, the weight is calculated on the basis of the 15% dye.

mg/l dye = mg/l of 15% solution added X 0.15

For both liquid and dry dye preparations, test solutions were prepared by weighing out the appropriate dye on an analytical balance (Mettler P 1200 or H20T).

TABLE II. INFORMATION ON 46 DYES

SELECTED FOR FISH BIOASSAY STUDIES

C.I. #	Color Index Name
10000-10999	Nitroso and Nitro Dyes and Indicators
10338	Disperse Yellow 42
11000-19990	Monoazo Dyes and Indicators
11855	Disperse Yellow 3
14645	Mordant Black 11
15510	Acid Orange 7 (Orange II)
15711	Acid Black 52
18965	Acid Yellow 17
19555	Direct Yellow 28
20000-35990	Diazo and Polyazo Dyes
20170	Acid Orange 24
20470	Acid Black 1
21010	Basic Brown 4 (Bismarck Brown)
22610	Direct Blue 6
24401	Direct Blue 218
24890	Direct Yellow 4
24895	Direct Yellow 12 (Chrysophenine)
25135	Acid Yellow 38
26360	Acid Blue 113
28160	Direct Red 81
29025	Direct Yellow 50
29160	Direct Red 23
30145	Direct Brown 95
30235	Direct Black 38
31600	Direct Black 80
40000-44990	Stilbene, Di- and Triarylmethane Dyes and Indicators
40000	Direct Yellow 11
40622	Fluorescent Brightening Agent 28
42000	Basic Green 4 (Malachite Green)
42535	Basic Violet 1 (Methyl Violet)
47000-47999	Quinoline Dyes, Fluorochromes, and Indicators
47020	Disperse Yellow 54
48000-49990	Polymethene, Thiazol, Indomine, and Indophenol Dyes
48055	Basic Yellow 11

C.I. #	Color Index Name
50000-52990	Azin, Oxazin and Thiazin Dyes
51005	Basic Blue 3
53000-Dyes from Natural Products and Organic Wastes	
53185	Sulfur Black 1
53630	Vat Blue 43
57000-74990	Hydroxyketone, Anthraquinone, Indigo, and Other Dyes
59105	Vat Orange 1
61505	Disperse Blue 3
61570	Acid Green 25
62055	Acid Blue 25
62500	Disperse Blue 7
63010	Acid Blue 45
67300	Vat Yellow 2
69015	Vat Brown 3
69500	Vat Green 3
69825	Vat Blue 6
74180	Direct Blue 86

Temperature for Tests

Temperature is known to affect response of fish to toxic materials (Alabaster, 1969; Cairns et al. 1971; Warren, 1971; Weiss and Botts, 1957). The testing laboratory was equipped with air conditioning, thermostatically-controlled heaters, and circulating fans in order to maintain constant (± 2° C) temperature. Although average water temperature in the various test series varied from 15 to 20° C, within any given test the temperature range did not exceed ± 2° C, as specified in Standard Methods (1971). Temperature was measured at the beginning, middle and end of the 96-hour exposure period with a thermistor probe (Yellow Springs Instrument Co.) or a common laboratory thermometer. Ambient temperature in the laboratory was monitored continuously with a recording thermometer (Tempscribe).

Dissolved Oxygen Content and Aeration of Test Solutions

Dissolved oxygen (DO) is required by fish for survival. Also DO is a factor in response of fish to toxic materials (Warren, 1971; Weiss and Botts, 1957). Standard Methods (1971) states that the DO should not drop below 5 mg/l in fish bioassay tests. However, fish can tolerate oxygen concentrations well below 3 mg/l, especially when maintained under laboratory conditions with restricted feeding (Doudoroff and Shumway, 1967).

Aeration of test solutions during static fish bioassay tests is not recommended if there is a possibility that such aeration may affect toxicity by accelerating loss of volatile materials. In order to avoid loss of components and yet maintain a safe dissolved oxygen level the following steps were taken:

(1) No feeding of fish was allowed during the test period
(2) Fish were starved for 48 hours prior to testing
(3) The experimental dilution water was allowed to equilibrate with the atmosphere prior to testing
(4) Dead fish were removed as soon as observed
(5) Aeration with compressed air was employed for only 5 minutes per jar after 48 hours of testing.

Dissolved oxygen concentration was measured initially and at 48-hour intervals during tests, using a polarographic oxygen analyzer (Yellow Springs Instrument Company).

pH Determination

pH was determined at the beginning, middle and end of the 96-hour exposure with a Leeds & Northrup pH meter equipped with combination

probe and expanded scale. No attempt was made to control pH during these tests; however, little variation in pH was noted.

Test Concentrations and Procedures

Small-scale exploratory bioassays were conducted to determine the range of concentrations to be tested in full-scale tests. For small scale tests, solutions were prepared with the following concentrations: 0.01, 0.1, 1.0, 10, and 100 mg/l. A test volume of 3.5 liters and 2-3 fish per container were used.

Based on results of small scale bioassays a full-scale test range was chosen, the concentrations falling between the highest concentration at which all fish survived and the lowest concentration at which all or most of the fish died. An exception was made for dyes in which all fish survived at 100 mg/l. In such cases 180 mg/l was highest concentration tested in the full-scale tests. It was assumed that higher concentrations were unrealistic and not likely to be encountered in streams receiving dye wastes.

In full-scale tests the TL_{50} was determined by testing a series of five concentrations, to enable more precise estimation. The series, chosen from Standard Methods (APHA et al. 1971), is based on progressive bisection of intervals on the logarithmic scale, i.e., 1.0, 1.8, 3.2, 5.6, and 10.0 mg/l, multiplied or divided as necessary by any power of 10. These values are evenly spaced when plotted on a logarithmic scale.

In each test series, control tests were conducted concurrently with the experimental dilution water alone. No more than 10% mortality occurred among control fish during the tests.

In most instances, 10 fish were used to test each experimental concentration. In some cases the number varied from 9 to 20 because of difficulties incurred by reduced visibility in solutions of high dye concentration.

Test containers were 5-gallon wide-mouth glass jars (Smith Container Corporation, Charlotte, N. C.), 25 cm (d) x 47 cm (h) and contained 15 liters of test solution. They were washed thoroughly with a cleaning solution (Micro, International Products Corporation), found to be effective in removing dyes. After washing, they were rinsed thoroughly with tap water, with acetone (if necessary), and finally with distilled water. During tests, the mouth of each container was covered with cheesecloth secured with a rubber band to prevent fish from jumping out of the container.

Fish were transferred from the stock tank to test containers with small-mesh dip nets or with wet hands.

In the stock tank fish were fed regularly (3-7 times per week). Fish were not fed for 48 hours prior to testing, nor for the duration of the tests.

Test Duration and Observations

Duration of all tests was 96 hours and fish were observed at 24-hour intervals. Any abnormal behaviour, number of dead fish, changes in appearance of test solution, etc., were noted. As soon as observed, dead fish were removed, weighed, and measured. At the end of the test, remaining fish were weighed and measured. Weighing and measuring of fish after, rather than before the test, reduced excessive handling which could have damaged or made them more susceptible to toxic compounds.

RESULTS AND DISCUSSION

Effects of the 46 dyes on fathead minnows were determined in 96-hour static bioassays at concentrations up to 180 mg/l. The TL_{50}, the concentration at which 50% of the experimental animals survive, was estimated by interpolation after plotting percentages of fish surviving at each concentration on semilogarithmic coordinate paper. Concentration was plotted on the logarithmic axis and percentage survival on the arithmetic axis, as shown for each dye on data sheets in the Appendix. To facilitate precise estimation of TL_{50}, the values reported actually were determined from larger graphs on high quality semilogarithmic paper, rather than directly on the data sheets.

A complete listing of the data is shown in Table III. The TL_{50} values for 29 dyes were higher than 180 mg/l; 3 others fell between 100 and 180 mg/l; and the remaining 14 were lower than 100 mg/l. The most toxic dyes were Basic Violet 1 (methyl violet), with a TL_{50} of 0.047 mg/l; Basic Green 4 (malachite green), and 0.12 mg/l; Disperse Blue 3, 1 mg/l; Basic Yellow 11, 3.2 mg/l; Basic Blue 3, 4 mg/l; Acid Blue 113, 4 mg/l; Basic Brown 4, 5.6 mg/l; Mordant Black 11, 6 mg/l; Acid Green 25, 6.2 mg/l; and Acid Black 52, 7 mg/l.

The two most toxic compounds, Basic Violet 1 and Basic Green 4, are triphenylmethane dyes. Malachite green (Basic Green 4) long has been used as a therapeutic fungicidal compound for fish (Innes, 1966; Lewis, 1963; Willford, 1966). It is an effective drug at appropriate concentrations, but is known to be toxic at higher concentrations. Willford (1966) tested 22 therapeutic compounds using six fishes (rainbow trout, brown trout, brook trout, lake trout, bluegills, and channel

TABLE III

EFFECT OF 46 SELECTED DYES ON THE FATHEAD MINNOW,

PIMEPHALES PROMELAS, IN STATIC BIOASSAY TESTS

C.I.#	Dye	TL_{50}, 96-hr., mg/l	Temp. °C
10338	Disperse Yellow 42	>180	15
11855	Disperse Yellow 3	>180	15
14645	Mordant Black 11	6	15
15510	Acid Orange 7	165	17
15711	Acid Black 52	7	15
18965	Acid Yellow 17	>180	17
19555	Direct Yellow 28	>180	15
20170	Acid Orange 24	130	17
20470	Acid Black 1	>180	15
21010	Basic Brown 4	5.6	20
22610	Direct Blue 6	>180	17
24401	Direct Blue 218	>180	17
24890	Direct Yellow 4	>180	15
24895	Direct Yellow 12	125	15
25135	Acid Yellow 38	23	15
26360	Acid Blue 113	4	15
28160	Direct Red 81	>180	17
29025	Direct Yellow 50	>180	17
29160	Direct Red 23	>180	17
20145	Direct Brown 95	>180	17
30235	Direct Black 38	>180	17
31600	Direct Black 80	>180	17
40000	Direct Yellow 11	>180	17
40622	Fluorescent Brightening Agent 28	>180	17
42000	Basic Green 4	0.12	18
42535	Basic Violet 1	0.047	15
47020	Disperse Yellow 54	>180	15
48055	Basic Yellow 11	3.2	18
51005	Basic Blue 3	4	15
53185	Sulfur Black 1	>180	15
53630	Vat Blue 43	>180	15
59105	Vat Orange 1	>180	15
59825	Vat Green 1	>180	15
61505	Disperse Blue 3	1	15
61570	Acid Green 25	6.2	18
62055	Acid Blue 25	12	15
62500	Disperse Blue 7	52	15
63010	Acid Blue 45	>180	15
67300	Vat Yellow 2	>180	15
69015	Vat Brown 3	>180	15
69500	Vat Green 3	>180	15
69825	Vat Blue 6	>180	15
74180	Direct Blue 86	>180	17
	Acid Yellow 151	29	15
	Disperse Red 60	>180	15
	Direct Yellow 106	>180	17

catfish) and found malachite green to be the most toxic, with a TL_{50} of 0.1-0.4 mg/l. He concluded that there was risk associated with long term treatment with malachite green at concentrations in excess of 0.11 mg/l. Lanzing (1965) stated that there was not significant difference in the oxalate and chloride forms of malachite green in regard to toxicity to whiting. In a recent survey of literature on effects of chemicals on aquatic life, numerous instances of malachite green toxicity to fish are cited, including:

(1) for rainbow trout, 18-day TL_{50} = 0.048 mg/l

(2) for rainbow trout, 2-5-day TL_{50} = 0.122 mg/l

(3) for channel catfish, 2-day TL_{50} = 0.14 mg/l (Kemp, Abrams, and Overbeck, 1971).

Malachite green has also been used as a bacteriostatic and amebicidic agent (Lillie, 1969). Indeed, the triphenylmethane dyes, as a group, affect the viability of bacteria (Albert, 1968; Salle, 1967) and, depending on concentration, may be bacteriostatic or bactericidal. Their relationship of concentration to toxicity is somewhat unusual. At low concentrations of crystal violet, bacteria show a non-logarithmic survivor curve, but at higher concentrations the survival curve is logarithmic in proportion to dye concentration (Hoffman and Rahn, 1944).

The antibacterial activity of triphenylmethane dyes is attributed to their cationic nature. It is suggested that the activity is due to "a reaction of the cation with some anionic groups of bacteria to give fully dissociated complexes" (Albert, 1968). Albert (1968) and co-workers showed that a quantitative relationship exists between

antibacterial action and ionization as cations in the acridine series. He also cites work by Goldacre and Phillips indicating strong correlation between ionization and antibacterial activity of the triphenylmethane dyes, Doebner's violet, malachite green, and brilliant green. He points out the dependence of ionization, and thus of antibacterial activity, on presence of chemically inert groups in the dye molecule.

No information has been found in the literature concerning the effect of Basic Violet 1 on fish. However, gentian violet, a similar dye, is used as a therapeutic agent for fish (Innes, 1966) and is an antibacterial agent.

The biological effects of Disperse Blue 3, Basic Yellow 11, Basic Blue 3, Acid Blue 113, Basic Brown 4, Mordant Black 11, Acid Green 25, and Acid Black 52 evidently are unknown.

In this study a substantial amount of time was devoted to seeking correlations between dye structure and toxicity to fish. These correlations are difficult to find, but several observations appear to be appropriate:

(1) None of the direct or vat dyes were toxic and most disperse dyes were not.

(2) Mordant Black 11 and Acid Black 52, similar to each other in structure, had similar TL_{50} values (6 and 7 mg/l, respectively).

(3) The triphenylmethane dyes were the most toxic of those tested. The triaminophenylmethane, Basic Violet 1, was toxic at a lower concentration than was the diaminophenylmethane, Basic Green 4.

(4) In the diaminoanthraquinone group, degree of toxicity appeared to be related to amount of substitution.

Since there were ten anthraquinone dyes in the group supplied by ADMI, it was possible to examine in some detail the relationship of anthraquinone structure to fish toxicity. Table IV shows the basic structure of aminoanthraquinones, the structure of several anthraquinone dyes tested in these experiments, and the TL_{50} for each dye.

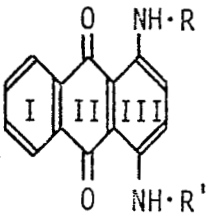
The amino groups present in aminoanthraquinone dyes vary in basicity. A methyl group attached to nitrogen, making a secondary amine, tends to strengthen the basicity; addition of a methyl group to a carbon atom also strengthens a base (Albert, 1968). Note in Table IV that the most toxic dye, Disperse Blue 3, has a methyl substituent on one amine group; that another toxic dye, Acid Green 25, has substitutions with methyl groups attached to carbon. Acid Blue 25 and Disperse Blue 7 have carbon-containing substituents on one or both amino groups.

The simple aminoanthraquinone dyes with no methyl or other carbon-containing substituents on the amino groups appeared to be harmless to fish, with TL_{50} values in excess of 180 mg/l. Included in this category are Acid Blue 45, a diaminoanthraquinone with one amino group on ring III and the second on ring I, and Disperse Red 60, with a single amino group.

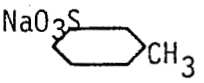
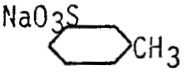
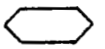
The complex aminoanthraquinone dyes (Vat Brown 3, Vat Green 3, Vat Blue 6, and Vat Yellow 2) all had TL_{50} values greater than 180 mg/l.

In order to define the relationship of anthraquinone structure and to determine whether <u>degree</u> of substitution into the basic structure

TABLE IV. ANTHRAQUINONE STRUCTURE AND FISH TOXICITY

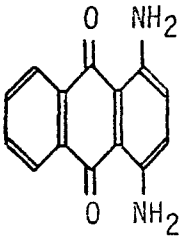


Primary Structure

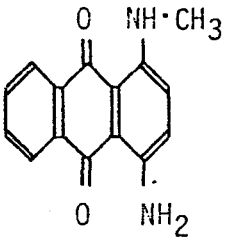
Dye	R	R'	I	II	III	TL_{50}, mg/l
(1) Disperse Blue 3	CH_3	CH_2CH_2OH	--	--	--	1
(2) Acid Green 25			--	--	--	6.2
(3) Acid Blue 25	H		--	--	SO_3Na	12
(4) Disperse Blue 7	C_2H_4OH	C_2H_4OH	OH,OH	--	--	52

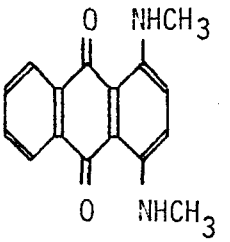
or type of substitution is the more important factor, it would be necessary to examine compounds with the following structures:

(1) diaminoanthraquinone, both amino groups on same ring



(2) diaminoanthraquinones, both amino groups on same ring, methylated





Overall, it may be concluded that the cationic dyes are the most likely to be toxic. This is not surprising, as it has been known since the 1920's that many organic cations are effective antibacterial agents. Indeed, much of the early work on antibacterial activity of organic cations involved the triphenylmethane dyes and the aminoacridines (Albert, 1968).

In view of this conclusion, it should be stressed that pH may affect toxicity by influencing degree of ionization of the dye and degree of ionization of its site of action on the test organism. In studies performed for ADMI, in which only dye samples and clean water were used, the pH remained near neutrality. If, in actual practice, dyes were discharged in conjunction with acid or alkaline materials

which would substantially change the pH, the toxic effect could be markedly changed. Commonly, however, dye waste streams receive primary and biological (secondary) treatment. As biological treatment demands a nearly neutral pH for effectiveness and since discharge of strongly acid or alkaline wastes to streams is not generally permitted, it would appear that marked variations in pH would not be expected.

SIGNIFICANCE OF STUDIES AND SUGGESTIONS FOR FURTHER RESEARCH

A 1936 "state-of-the-art" survey of textile waste treatment described dye wastes as large in volume, high in color and, in many instances, toxic (Geyer and Perry, 1936). Sulfur dyes and aniline black dyes were especially incriminated for their effects on sewage treatment plant and receiving stream biota. More than 25 years later another review of toxic effects of dyes concluded

> "Despite the fact that dyes are used extensively in industry and despite the fact that numerous references state that dyes are toxic substances, relatively few specific references dealing with the toxicity of dyes were uncovered in this survey." (McKee and Wolfe, 1963).

Today, nearly a decade since that review, the same statement may be made again, as evidenced in the review of Kemp et al (1971) and Harrison and Bryan (1971).

The research described in this report constitutes the first extensive study of effects of dyes on fish. The 46 samples which were used in short-term static bioassays were selected and especially prepared, by members of ADMI, to be representative of widely-used groups of dyes.

The data indicate that many dyes should present no practical problem from the point of view of toxicity to fish because their threshold values are far above concentrations which could be acceptable in streams, considering regulatory limitations on colors. On the

other hand, data for others suggest the possibility of environmental problems relating to fish toxicity. These include dyes for which the observed TL_{50} concentrations, reduced by customary "application factors" to yield probable safe levels, result in concentrations below those which would cause objectionable color in streams. With these, it is possible that toxicity to fish could be a problem, even at concentrations lower than those deemed objectionable in the stream for reasons of appearance.

Wastewaters containing dyes may pass into the environment in practice via municipal sewerage systems or through direct discharge to streams by industries using them. In either event, pollution control regulations today require that virtually all wastewaters discharged by either route must be subjected to specified types of treatment before reaching the stream. In many instances, the dye may be removed by a wastewater treatment process or modified to produce a less toxic form. This might be accomplished through attachment of the dye to suspended materials removed during primary treatment or to growths developed in the biological system. This is reasonable to anticipate as a possibility because some of the dyes with relatively low TL_{50} values have been used as biological stains because of strong tendencies to combine with cell materials.

In view of this situation, it is logical to anticipate the toxicity of some of the dyes could be substantially less in practice than suggested by the rather simplistic tests conducted during these preliminary investigations. It is recommended that further studies

be undertaken of those dyes showing significant toxicity in the preliminary tests. The procedure could be based on mixture of the dye with municipal sewage and treatment of the blend by a sequence of processes which reasonably could be found in a practical system. Influent and treated effluent could be subjected to determination of TL_{50} to find "before" and "after" toxicity to fish.

A proposal has been submitted to ADMI outlining those proposed investigations in more detail.

REFERENCES

Alabaster, J. S. 1969. The Comparative Sensitivity of Coarse Fish and Trout to Pollution. Proc. of Fourth British Coarse Fish Conference, Liverpool Univ., March 27-29.

Albert, Adrien. 1968. Selective Toxicity, 4th ed. Methuen & Co., Ltd., London.

APHA, AWWA. WPCF 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed. American Public Health Assn., Inc., New York.

Brown, V. M. 1969. The Prediction of the Acute Toxicity of River Waters to Fish. Proc. of the Fourth British Coarse Fish Conference, Liverpool Univ., March 27-29.

Cairns, J., Jr. 1969. Fish Bioassays - Reproducibility and Rating. Revista de Biologia 7 (1-2): 7-12.

Cairs, J., Jr., T. K. Bahns, D. T. Burton, K. L. Dickson, R. E. Sparks, and W. T. Waller. 1971. The Effects of pH, Solubility and Temperature Upon the Acute Toxicity of Zinc to the Bluegill Sunfish (Lepomis machrochirus Raf.). Trans. Kansas Acad. Science 74: 81-92.

Cairns, J., Jr. and A. Scheier. 1968. A Comparison of the Toxicity of Some Common Industrial Waste Components Tested Individually and Combined. Prog. Fish - Culturist 30: 3-8.

Carmichael, C. and S. R. Cocket. 1968. "Dyes and Dyeing." Encyclopedia Brittanica, Vol. 7, p. 814.

Doudoroff, P. and D. L. Shumway. 1967. Dissolved Oxygen Criteria for the Protection of Fish. Am. Fisheries Soc., Special Publication No. 4, 13-19.

Flege, R. K. 1970. Determination of Degraded Dyes and Auxiliary Chemicals in Effluents from Textile Dyeing Processes. Environmental Resources Center, Georgia Institute of Technology, Atlanta. [Abstr. in Harrison, P. S., and C. E. Bryan. 1971. A Literature Survey of Colored Wastes, American Dye Manufacturers Institute, Inc.].

Geyer, J. C., and W. A. Perry. 1936. Textile Waste Treatment and Recovery. The Textile Foundation, Inc., Washington, D. C.

Hoffmann, C. E., and O. Rahn. 1944. The Bactericidal and Bacteriostatic Action of Crystal Violet. J. Bact. 47: 177-186.

Innes, W. T. 1966. Exotic Aquarium Fishes (The Innes Book), 19th ed. Metaframe Corporation, Division of Mattel, Inc., Maywood, New Jersey.

Kemp, H. T., J. P. Abrams, and R. C. Overbeck. 1971. Effect of Chemicals on Aquatic Life - Water Quality Criteria Data Book, Vol. 3, 528 pp. Water Pollution Control Research Series. 1805GWV05/71, Environmental Protection Agency, Washington, D. C.

anzing, W. J. R. 1965. Observations on Malachite Green in Relation to Its Application to Fish Diseases. Hydrobiologia 25 (3/4): 426-441.

ewis, W. M. 1963. Maintaining Fishes for Experimental and Instructional Purposes. Southern Illinois University Press, Carbondale.

illie, R. D. 1969. H. J. Conn's Biological Stains, 8th ed. The Williams & Wilkins Company. Baltimore.

cKee, J. E., and H. W. Wolf. 1963. Water Quality Criteria, 2nd ed., Publ. No. 3-A, State Water Quality Control Board. Sacramento, California., 548 pp.

orter, J. J. 1971. State of the Art of Textile Waste Treatment. Water Pollution Control Research Series 12090 ECS 02/71, Environmental Protection Agency, Washington, D. C.

alle, A. J. 1967. Fundamental Principles of Bacteriology, 6th ed. McGraw-Hill Book Company, New York.

esarcik, J. and J. Smisik. 1967 Vliv aplikace technicke malachitove zelene na chemismus vody, bentickou rybnicni zvirena a p razito fauna kapriko pludku [Influence of the application of commercial malachite green on the chemical condition of water and benthic pond fauna]. Pr. Vyzk. Ustava Rybarskeho a Hydrobiolog. Vodn. 7: 33-57. Bibliogr. Abstract 391 in Bibliography of Aquaculture, Publ. 71-4 (1971), Coastal Plains Center for Marine Development Services, Wilmington, N. C. 28401.

sai, Chu-Fa. 1971. Water Quality Criteria to Protect the Fish Population Directly Below Sewage Outfalls. Completion Report, B-006-Md, 14-01-001-1910, Maryland Water Resources Research Center.

arren, C. E. 1971. Biology and Water Pollution Control. W. B. Saunders Company, Philadelphia.

eiss, C. M., and J. L. Botts. 1957. Factors Affecting the Response of Fish to Toxic Materials. Sewage and Industrial Wastes 29: 810-818.

illford, W. A. 1966. Toxicity of 22 Therapeutic Compounds to 6 Fishes. Resource Publication 18, U. S. Dept. of Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, 10 pp.

APPENDIX A

Dye Sample Preparation for ADMI Study

he samples of dyes provided by the ADMI for environmental studies are ll composite samples from a variety of manufacturers. Following is a escription of how the samples were prepared.

he list of dyes selected for evaluation was circulated to the member ompanies of ADMI with an invitation to provide as many as possible of he dyes on the list before a deadline date. The instructions provided he manufacturers asked that the Acid, Basic, Direct, Mordant Black 11 nd Fluorescent Brightening Agent 28 samples be dried and ground concen- rate with no additives present; the Disperse and Vat samples be finished, et presscake with no additives present and that the Sulfur Black 1 be olution with no additives present. The presscakes of dyes as produced n ordinary manufacture vary considerably with respect to moisture con- ent and are filtered from solutions that contain amounts of salts, specially sodium chloride and sodium sulfate, that vary from almost one to saturated. For this reason the organic dye content of dried resscake will vary from about 100% to about 50% in the case of very wet astes taken from saturated salt solutions.

ne samples collected by the manufacturers were then sent to designated ollecting points as follows:

. Acid and Basic dyes - GAF
. Direct, Mordant and Fl. Brightening - Verona
. Vat and Sulfur dyes - Sodyeco
. Disperse dyes - DuPont.

qual quantities from each of the collected samples of the Acid, Basic, irect, Mordant and Fluorescent Brightening Agent were found and blended o provide the samples used for study.

qual quantities of the vat dye pastes were mixed together and an amount f Tamol SN (dispersing agent) equal to the weight of the active ingredi- nt of dye was added. The mixture was then milled to provide a reasonable ispersion to contain 15% active ingredient (dye) and 15% Tamol SN. This aterial was used to provide the samples for study.

ne disperse dye samples were prepared in the same manner as the vat dye amples excepting that Reax 85A was used for the dispersing agent.

eax 85A is a lignin sulfonate and Tamol SN is a condensed naphthalene ulfonic acid. Samples of the same lot of materials used to make the ispersions for study have been retained.

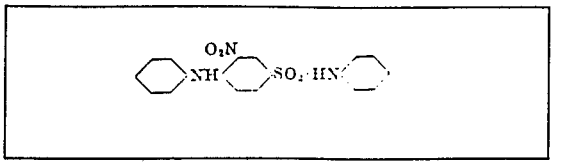
ortions of the blended dye samples as provided for study have been etained for possible future use.

2/6/71 Correspondence from R.H. HORNING, Dyes and Chemicals Division, Crompton & Knowles Corporation, Reading, Pennsylvania.

UNC WASTEWATER RESEARCH CENTER

FISH BIOASSAY TESTS

AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Disperse Yellow 42 C. I. No. 10338 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.4 g; range, 1.0 to 4.3 g. Avg. length 6.5 cm; range, 4.4 to 8.0 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

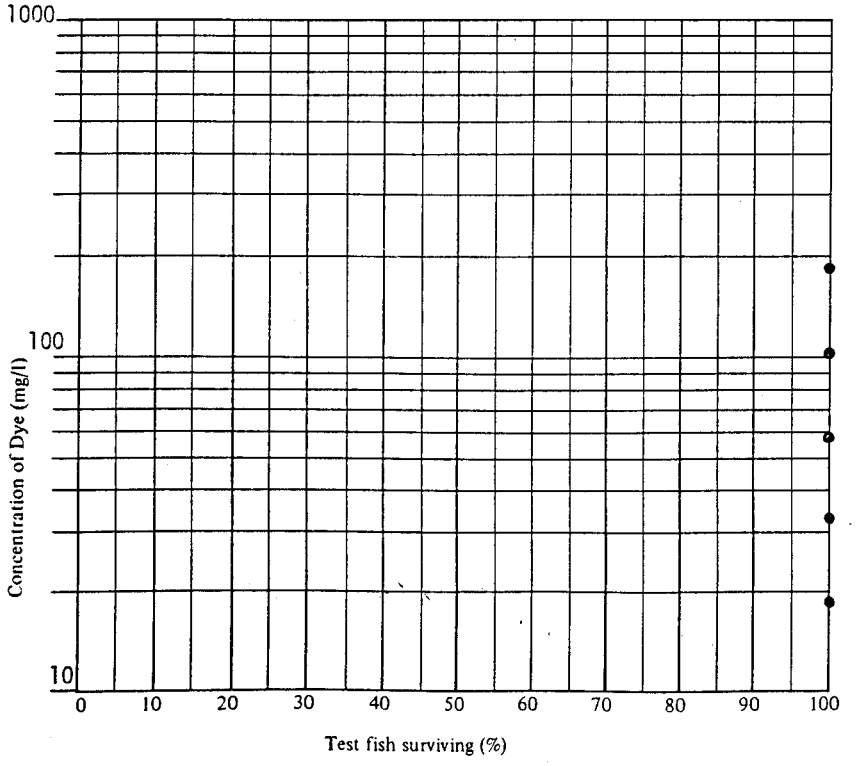
   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.0 to 17.2 C. Initial dissolved oxygen 7.4-7.8 mg/l; range during test, 1.7 to 7.8 mg/l. Initial pH 7.1-7.9 ; range during test, 6.6 to 7.9 . (Aerated 5 min each at 48 hr.)

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

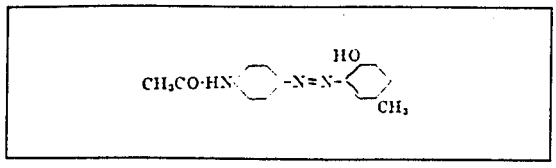
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.2	7.5	-	3.5	6.8	2.1
32	7.4	7.4	-	2.7	6.8	2.0
56	7.4	7.7	-	2.2	7.0	2.4
100	7.5	7.7	-	1.9	6.9	1.7
180	7.9	7.8	-	1.8	6.9	1.7

Observations on behavior of fish during the tests: Fish dyed faint yellow at 180 mg/l.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Disperse Yellow 3 C. I. No. 11855 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.1 g; range, 1.0 to 3.7 g. Avg. length 6.3 cm; range, 4.9 to 7.6 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

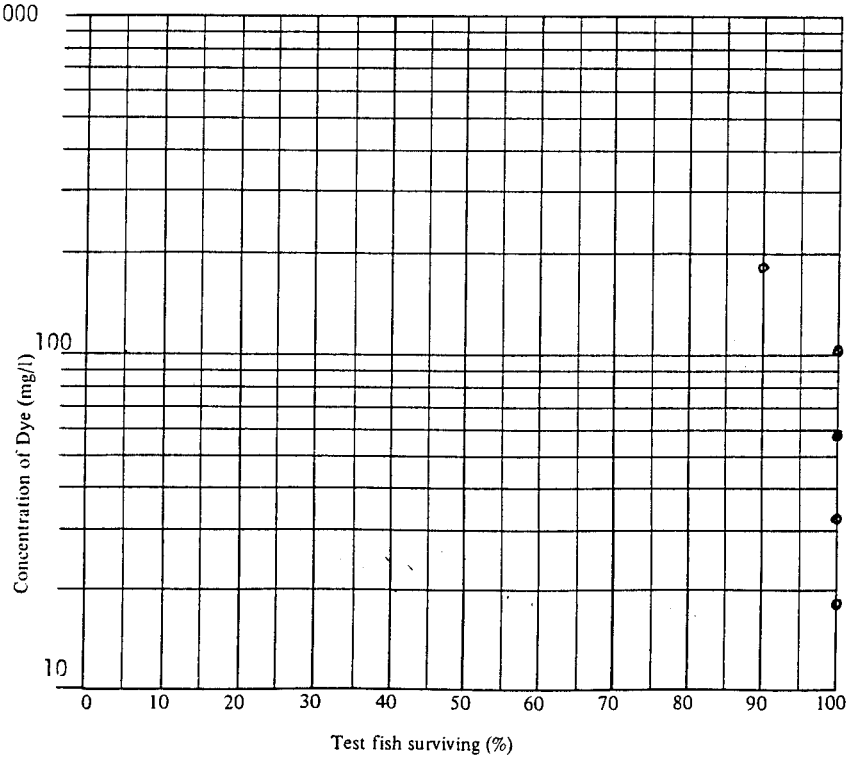
   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.3 to 16.8 C. Initial dissolved oxygen 7.3-7.6 mg/l; range during test, 1.6 to 7.6 mg/l. Initial pH 7.1-7.8 ; range during test, 6.6 to 7.8 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: >130 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	9

TEST CONDITIONS

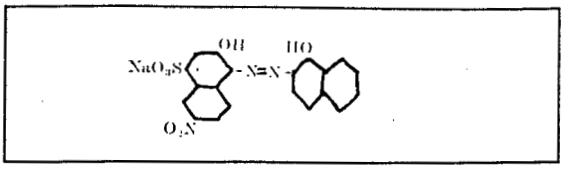
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.3	7.4	-	2.5	6.8	2.7
32	7.4	7.5	-	3.0	6.8	2.1
56	7.4	7.3	-	3.4	6.8	1.8
100	7.6	7.4	-	4.0	6.9	2.0
180	7.8	7.5	-	3.1	6.9	1.6

Observations on behavior of fish during the tests: Fish dyed yellow. Yellow droppings were observed in containers.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Mordant Black 11 C. I. No. 14645 Date tested 1/31/72

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Stored at c. 20 C in dark

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 2.5 g; range, 1.14 to 5.37 g. Avg. length 6.6 cm; range, 5.10 to 8.20 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.9 ; TOC 2 mg/l; Fe <0.2 mg/l;

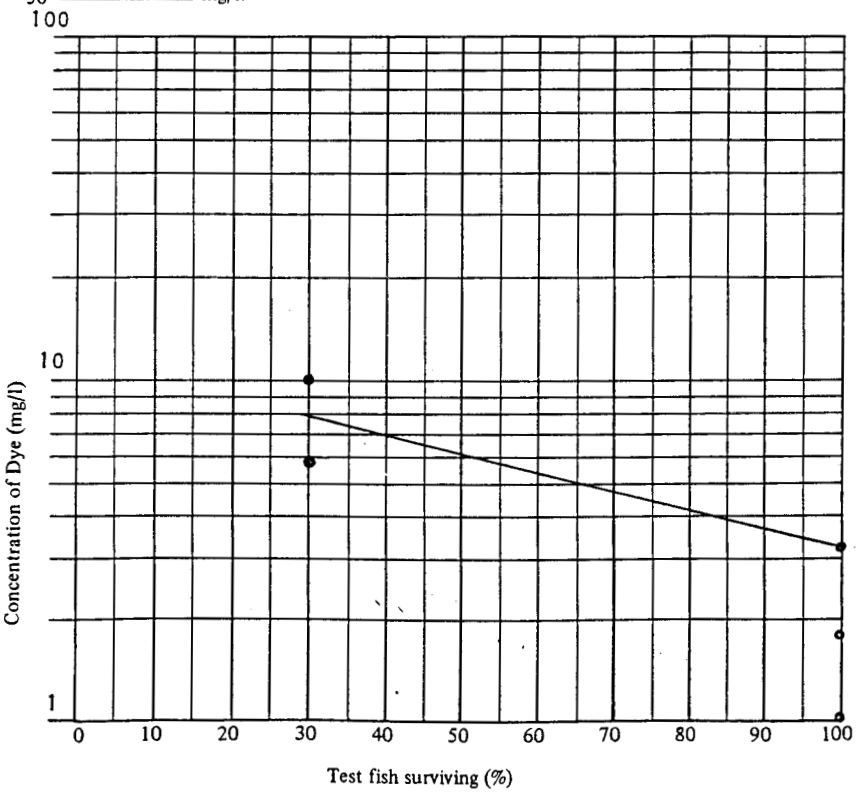
   Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l;

   turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.0 to 17.1 C. Initial dissolved oxygen 6.6-7.1 mg/l; range during test, 2.2 to 7.1 mg/l. Initial pH 6.9-7.0 ; range during test, 6.2 to 7.0 . (Aerated for 5 min each at 48 hr)

7. 96-hour TL_{50}: 6 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
1.0	10	10	10	10	10
1.8	10	10	10	10	10
3.2	10	10	10	10	10
5.6	10	7	3	3	3
10	10	5	3	3	3

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.92	7.0	6.2	2.7	6.5	2.6
1.0	6.98	7.1	6.25	2.5	6.5	2.3
1.8	6.98	6.9	6.3	2.2	6.6	2.6
3.2	6.98	6.7	6.3	2.2	6.7	2.5
5.6	6.97	6.7	6.4	2.7	6.7	2.5
10	6.99	6.6	6.4	2.6	6.7	2.6

Observations on behavior of fish during the tests: Surviving fish dyed pale purple at 5.6 mg/ℓ and 10 mg/ℓ

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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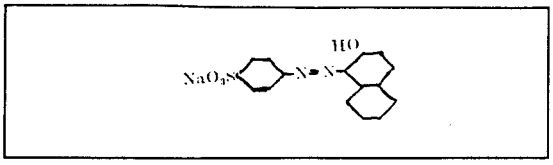
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Acid Orange 7 C. I. No. 15510 Date tested 3/2-3/6/72

   Supplier American Dye Manufacturers Inst. Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10-11

   Avg. wt. 0.55 g; range, 0.21 to 0.95 g. Avg. length 4.17 cm; range, 3.10 to 5.20 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

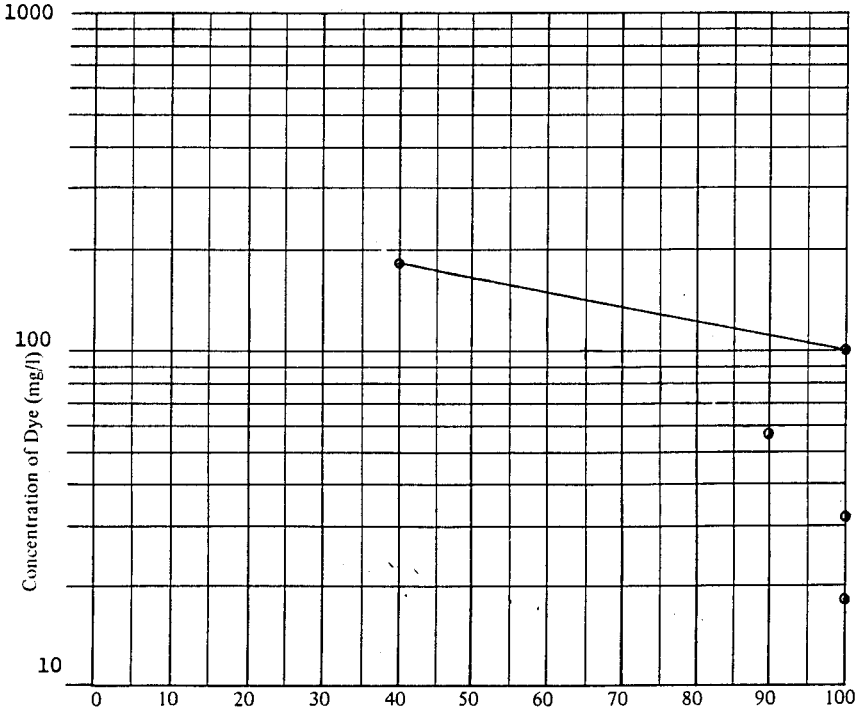
   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 6.5 mg/l; total dissolved solids 85 mg/l;

   turbidity 0 JTU; total alkalinity 19 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.1 to 18.4 C. Initial dissolved oxygen 6.6-8.1 mg/l; range during test, 5.0 to 7.4 mg/l. Initial pH 6.4 ; range during test, 6.4 to 7.3 .

7. 96-hour TL_{50}: 165 mg/l. (Aerated 5 min. each at 48 hr.)



Acclimation of fish Fish received 2/14/72 maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C.

Pretreatment of fish Treated with Tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	9	9	9
100	11	11	11	11	11
180	10	10	9	8	4

TEST CONDITIONS

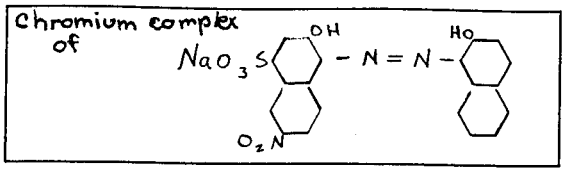
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.4	8.1	6.6	6.9	7.0	6.4
18	6.4	7.2	6.8	5.4	7.2	6.0
32	6.4	7.8	6.8	5.7	7.3	6.3
56	6.4	6.6	6.8	5.9	7.2	6.5
100	6.4	7.3	6.8	5.0	7.2	6.5
180	6.4	7.4	6.8	5.5	7.1	5.9

Observations on behavior of fish during the tests: Living fish dyed pale orange.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Acid Black 52 C. I. No. 15711 Date tested 1/31/72

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Stored at c. 20 C in dark

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 9-10

   Avg. wt. 2.5 g; range, 1.07 to 4.65 g. Avg. length 6.6 cm; range, 5.70 to 8.20 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.9 ; TOC 2 mg/l; Fe <0.2 mg/l;

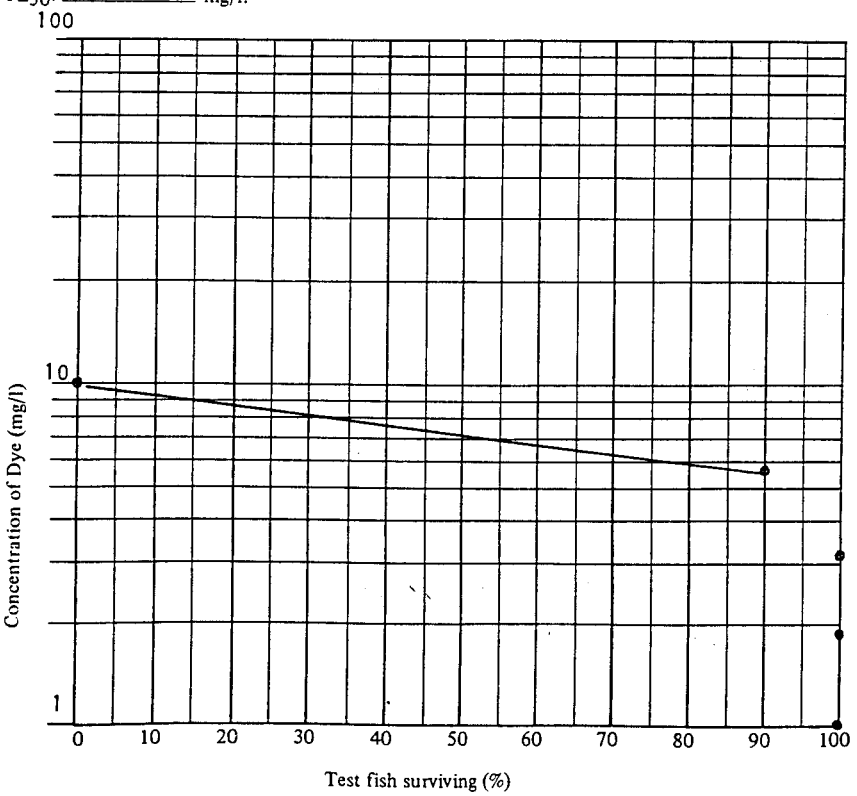
   Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l;

   turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.1 to 17.1 C. Initial dissolved oxygen 7.1-7.5 mg/l; range during test, 1.6 to 7.5 mg/l. Initial pH 6.9-7.1 ; range during test, 6.2 to 7.1 . (Aerated for 5 min each at 48 hr)

7. 96-hour TL_{50}: 7 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
1	10	10	10	10	10
1.8	10	10	10	10	10
3.2	9	9	9	9	9
5.6	10	9	9	9	9
10	10	2	0	-	-

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.9	7.0	6.2	2.7	6.5	2.6
1	7.0	7.1	6.3	1.9	6.7	2.8
1.8	7.1	7.3	6.3	1.6	6.7	2.2
3.2	7.1	7.5	6.4	2.5	6.6	3.0
5.6	7.1	7.4	6.35	2.0	6.7	2.6
10	7.1	7.5	6.4	2.0	-	-

Observations on behavior of fish during the tests: Fish were not dyed.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

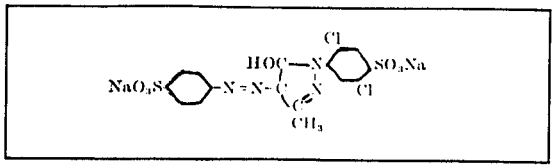
Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

Form No. 2-3

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Acid Yellow 17 C. I. No. 18965 Date tested 3/2-3/6/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.55 g; range, 0.31 to 0.87 g. Avg. length 4.19 cm; range, 3.40 to 5.00 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 6.5 mg/l; total dissolved solids 85 mg/l;

   turbidity 0 JTU; total alkalinity 19 mg/l as $CaCO_3$.

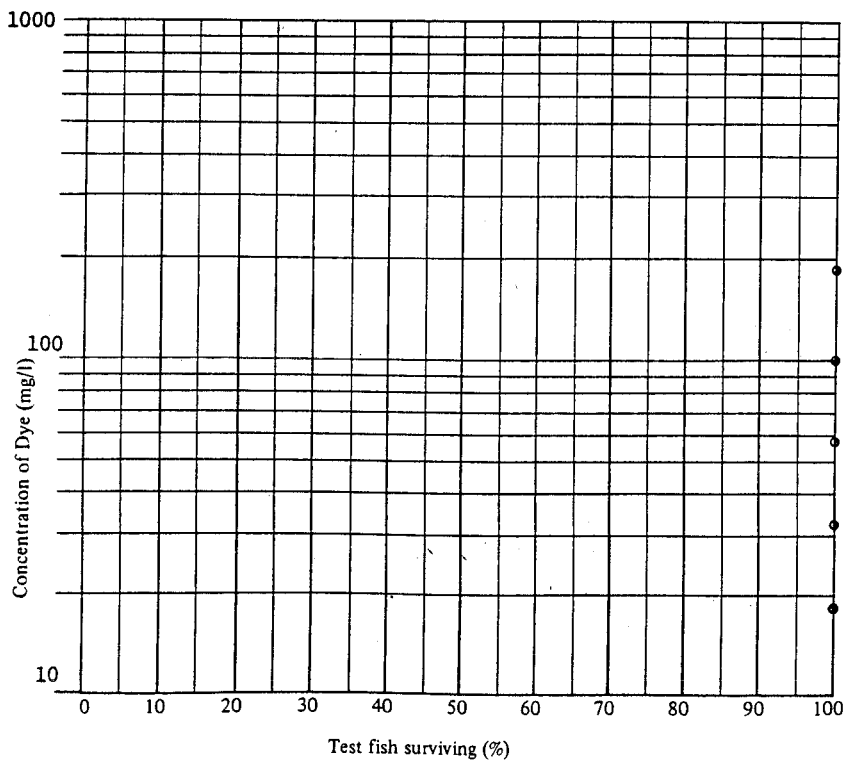
5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.0 to 18.3 C. Initial

   dissolved oxygen 7.1-8.1 mg/l; range during test, 5.3 to 7.4 mg/l. Initial pH 6.4-7.0 ;

   range during test, 6.4 to 7.2 .

7. 96-hour TL_{50}: >180 mg/l. (Aerated 5 min. each at 48 hr.)



Acclimation of fish Fish received 2/14/72 maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water 14-18 C.

Pretreatment of fish Treated with tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.4	8.1	6.6	6.9	7.0	6.4
18	6.7	7.2	6.8	5.7	7.2	6.0
32	6.7	7.2	6.7	6.0	7.2	6.2
56	6.9	7.4	6.7	5.3	7.1	6.3
100	7.0	7.1	6.8	5.5	7.1	6.1
180	7.0	7.2	6.8	5.4	7.1	6.4

Observations on behavior of fish during the tests: Not remarkable

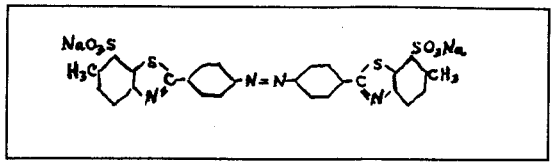
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Yellow 28 C. I. No. 19555 Date tested 3/6-3/10/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.69 g; range, 0.30 to 1.91 g. Avg. length 4.32 cm; range, 3.50 to 6.00 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

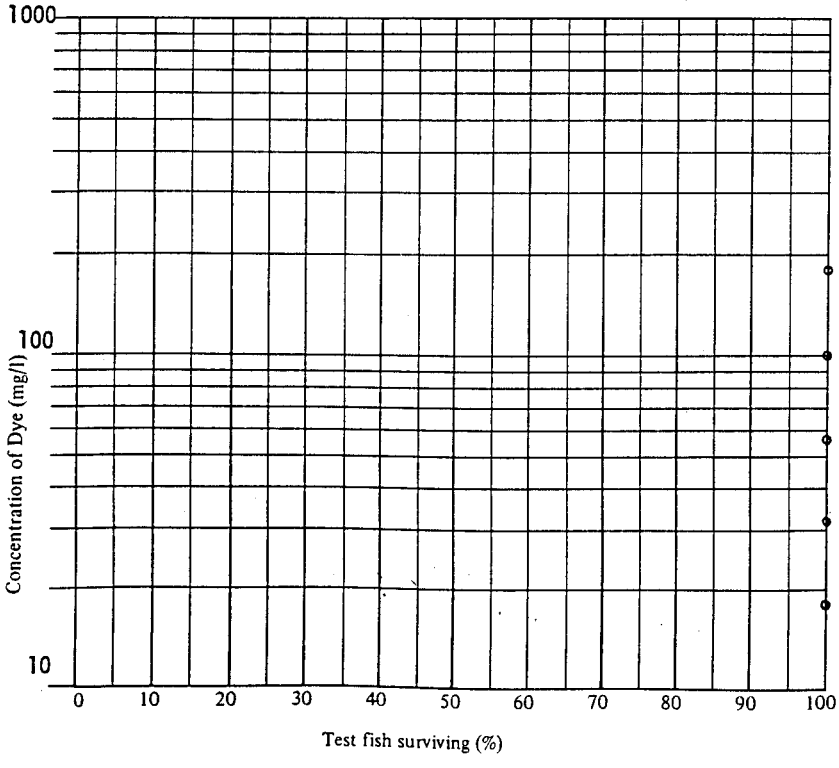
   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 5.5 mg/l; total dissolved solids 59 mg/l;

   turbidity 0 JTU; total alkalinity 15 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 13.2 to 17.2 C. Initial dissolved oxygen 7.1-7.5 mg/l; range during test, 5.5 to 7.5 mg/l. Initial pH 6.9-7.5 ; range during test, 6.8 to 7.5 .

7. 96-hour TL_{50}: >180 mg/l. (Aerated 5 min each at 48 hr).



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C.

Pretreatment of fish Treated with tetracycline HCl (Tetrachel) 2/14/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.92	7.5	6.81	6.2	6.70	6.6
18	7.28	7.3	6.87	5.5	6.81	6.1
32	7.30	7.2	6.92	5.8	6.86	6.6
56	7.38	7.1	7.05	6.4	6.87	6.4
100	7.42	7.3	7.11	6.1	6.85	6.6
180	7.50	7.2	7.21	6.4	6.88	6.4

Observations on behavior of fish during the tests: Not remarkable

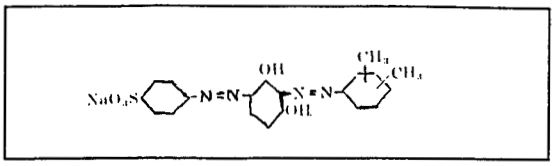
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Acid Orange 24 C. I. No. 20170 Date tested 3/2-3/6/72

   Supplier American Dye Manufacturers Inst. Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C.

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 9-11

   Avg. wt. 0.58 g; range, 0.27 to 1.48 g. Avg. length 4.24 cm; range, 2.70 to 6.00 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

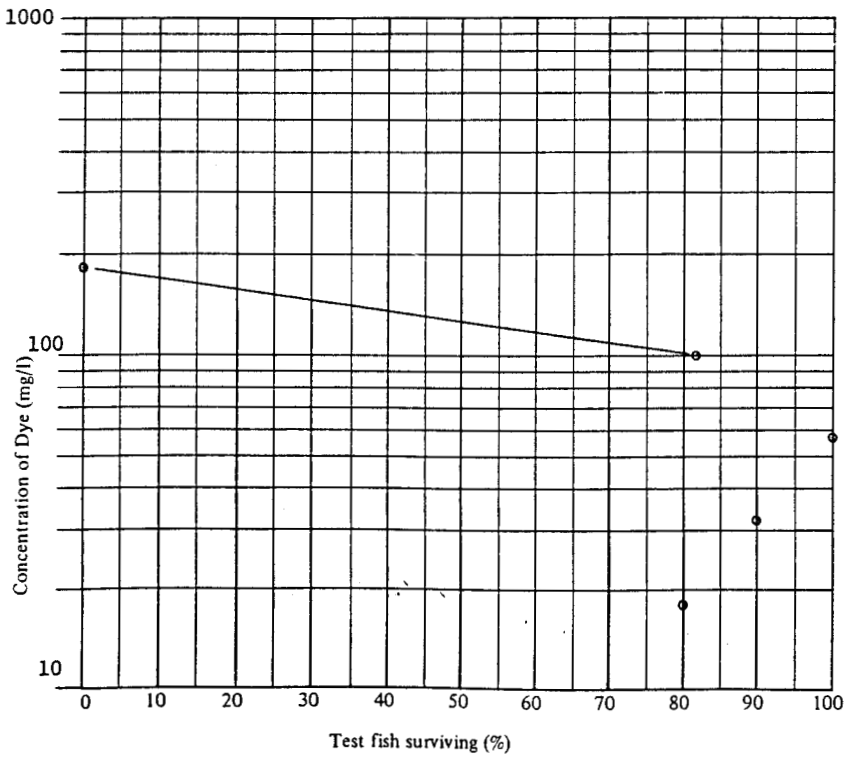
   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 6.5 mg/l; total dissolved solids 85 mg/l;

   turbidity 0 JTU; total alkalinity 19 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.2 to 18.6 C. Initial dissolved oxygen 5.7-9.0 mg/l; range during test, 5.5 to 9.0 mg/l. Initial pH 6.2-6.6 ; range during test, 6.2 to 7.2 . (Aerated 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 130 mg/l.



Acclimation of fish Fish received 2/14/72 ;maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C.

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	9	-	8
32	10	9	9	9	9
56	10	10	10	10	10
100	11	11	11	9	9
180	9	9	8	5	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.4	8.1	6.6	6.9	7.0	6.4
18	6.2	8.6	6.7	5.9	7.1	6.7
32	6.2	9.0	6.8	5.6	7.0	6.2
56	6.6	8.6	6.8	5.6	7.2	6.2
100	6.6	6.7	6.8	5.7	7.3	6.4
180	6.5	5.7	6.8	5.5	7.3	6.3

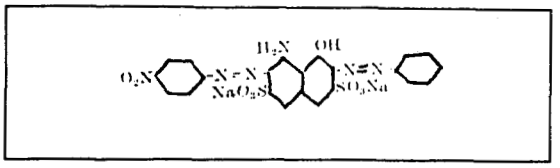
Observations on behavior of fish during the tests: Living fish dyed yellow to pale orange.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Acid Black 1 C. I. No. 20470 Date tested 3/23-3/27/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10-15

   Avg. wt. 0.56 g; range, 0.26 to 0.96 g. Avg. length 4.05 cm; range, 3.40 to 4.80 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8; TOC 4 mg/l; Fe <0.02 mg/l;

   Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l;

   turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

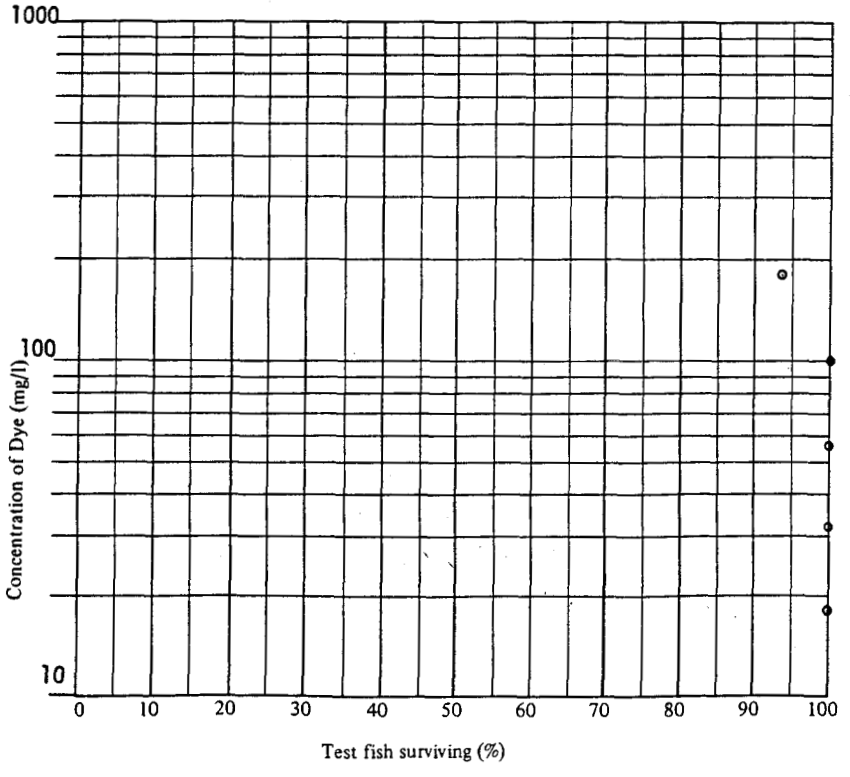
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.0 to 17.3 C. Initial

   dissolved oxygen 6.2-7.0 mg/l; range during test, 5.0 to 7.0 mg/l. Initial pH 7.1-7.7;

   range during test, 6.5 to 7.7.

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	-	-	-	9
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	15	-	-	-	14

TEST CONDITIONS

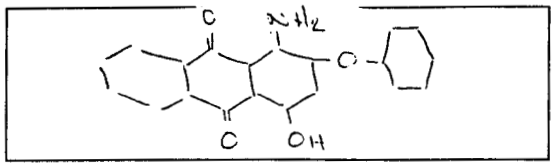
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.1	6.2			6.5	5.0
32	7.2	6.5			6.7	5.2
56	7.3	6.6			6.7	5.5
100	7.3	6.8			6.7	5.1
180	7.7	7.0			6.7	5.4

Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Disperse Red 60 C. I. No. - Date tested Jan. 6-10, 1972

   Supplier ADHI Form supplied Dispersion of 15% (wt) with Reax 83-A (15%)

   Conditions of storage refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.5 g; range, 1.2 to 4.8 g. Avg. length 6.7 cm; range, 5.2 to 8.3 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water charcoal and sand filtered Chapel Hill tap Water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 0 mg/l; Fe <0.02 mg/l;

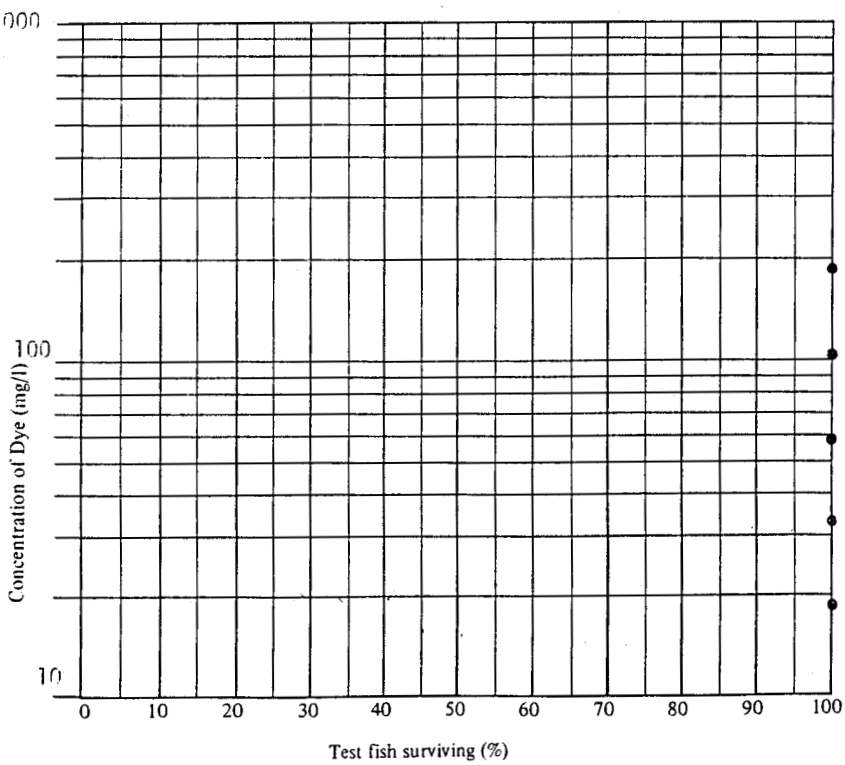
   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.2 to 17.1 C. Initial dissolved oxygen 7.3-7.6 mg/l; range during test, 1.1 to 7.6 mg/l. Initial pH 7.1-7.5 ; range during test, 6.8 to 7.5 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
10	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
10	7.4	7.3	-	2.2	6.8	1.9
32	7.5	7.5	-	1.5	6.8	1.7
56	7.5	7.4	-	1.2	6.8	1.1
100	7.5	7.4	-	1.6	6.8	1.5
180	7.3	7.4	-	2.5	6.8	2.7

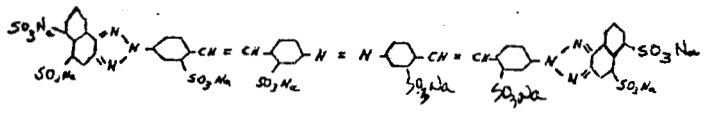
Observations on behavior of fish during the tests: Fish were dyed pink at 100 mg/l and 180 mg/l. Red droppings observed in containers.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Yellow 106 C. I. No. - Date tested 11/11-15/71

   Supplier ADMI Form supplied Composite, dry powder

   Conditions of storage Composite stored at 20°C in dark. Stock solutions refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.2 g; range, 0.4 to 2.6 g. Avg. length 5.0 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

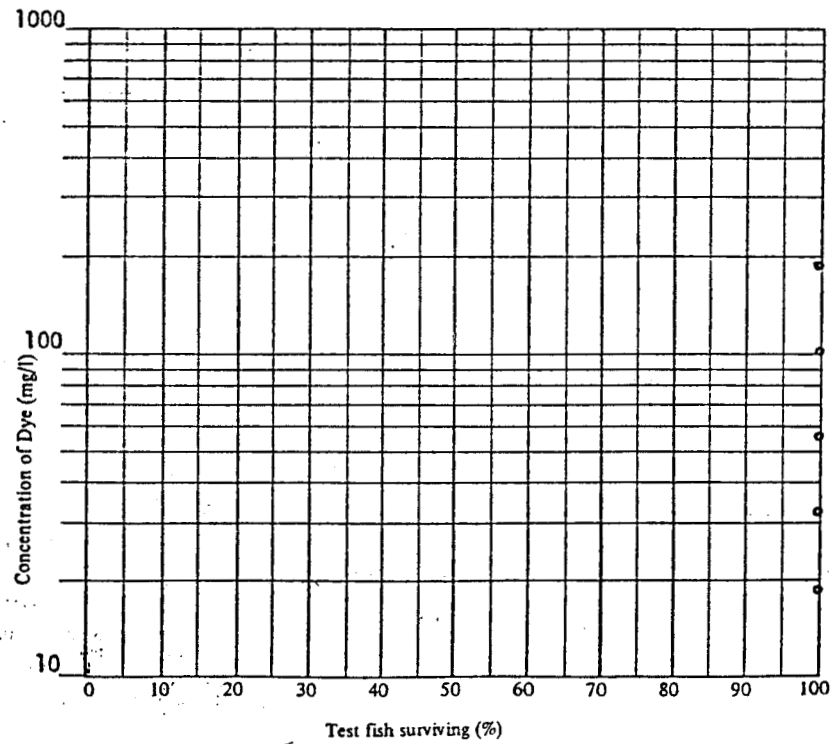
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.5 to 17.5 C. Initial dissolved oxygen 7.1-7.5 mg/l; range during test, 2.7 to 7.5 mg/l. Initial pH 7.0-7.3 ; range during test, 6.9 to 7.3 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air.

Pretreatment of fish Treated with tetracycline Oct. 20-22; NaCl 1l/l; tetracycline 1l/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.0	7.6	7.0	3.1	6.8	2.1
32	7.0	7.6	7.1	3.6	7.0	3.7
56	7.2	7.5	7.2	3.7	7.0	3.8
100	7.2	7.5	7.2	3.9	7.1	3.7
180	7.3	7.5	7.3	3.6	7.1	3.6

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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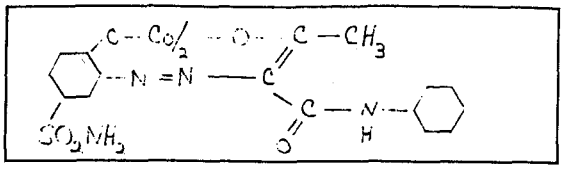
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Acid Yellow 151 C. I. No. - Date tested 1/31/72

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Stored at c. 20 C in dark

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 2.5 g; range, 0.82 to 4.78 g. Avg. length 6.5 cm; range, 3.90 to 8.20 cm.

3. Test jars: Material Glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

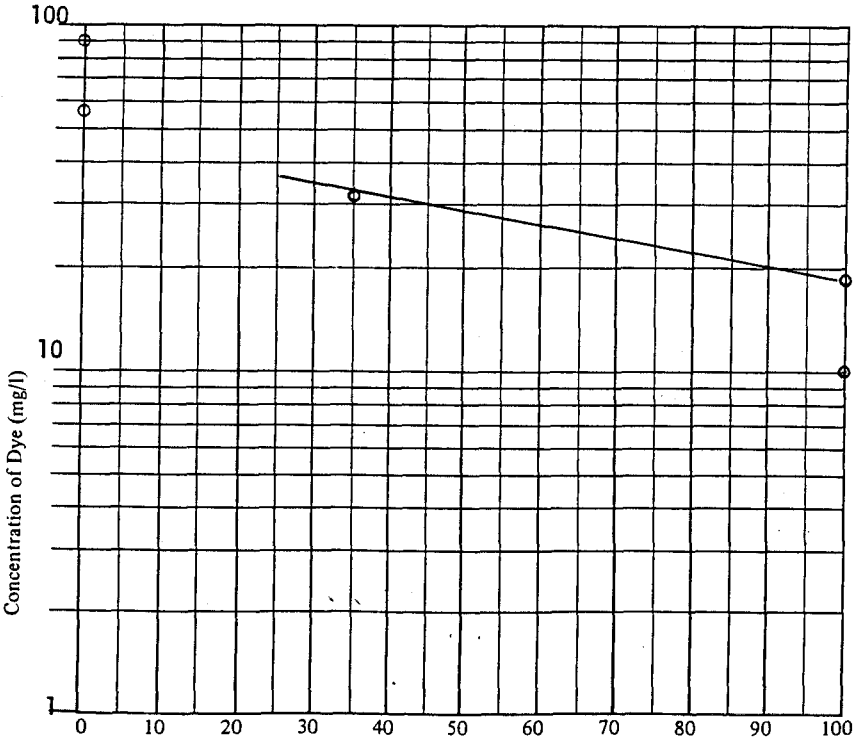
4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.9; TOC 2 mg/l; Fe <0.2 mg/l; Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l; turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.0 to 17.0 C. Initial dissolved oxygen 7.3-7.5 mg/l; range during test, 2.0 to 7.5 mg/l. Initial pH 7.3-7.6; range during test, 6.2 to 7.5. (Aerated for 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 29 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
10	10	10	10	10	10
18	10	10	10	10	10
32	10	4	4	4	4
56	10	0	-	-	-
100	10	0	-	-	-

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.3	7.5	6.2	2.6	6.5	2.7
10	7.5	7.4	6.4	2.6	6.7	2.2
18	7.4	7.5	6.3	2.2	6.6	2.0
32	7.4	7.3	6.4	2.7	6.8	2.6
56	7.4	7.4	7.1	7.0	--	--
100	7.6	7.4	7.1	7.2	--	--

Observations on behavior of fish during the tests: Fish dyed very pale yellow. All fish died within 6 hr at 56 mg/ℓ and 100 mg/ℓ

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

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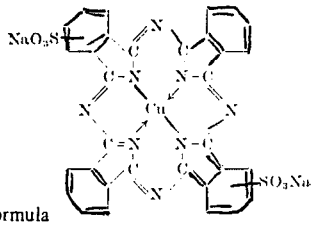
UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Blue 86 C. I. No. 74180 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.1 g; range, 0.3 to 2.6 g. Avg. length 4.9 cm; range, 3.0 to 6.5 cm.

3. Test jars: Material glass ; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

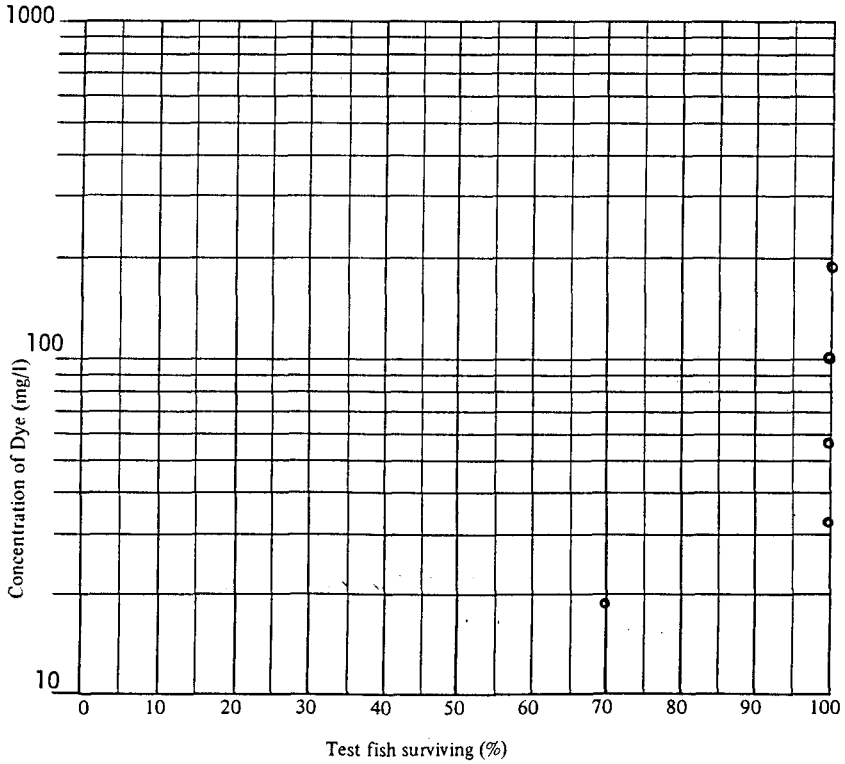
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 17.0 to 17.7 C. Initial dissolved oxygen 7.4-7.5 mg/l; range during test, 2.8 to 7.5 mg/l. Initial pH 7.1-7.2 ; range during test, 6.7 to 7.2 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22, NaCl 1l/l; tetracycline 1l/2. Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	7
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.1	7.4	7.2	3.9	6.8	3.9
32	7.1	7.5	7.2	3.8	6.7	3.6
56	7.2	7.5	7.2	3.9	6.7	3.4
100	7.1	7.5	7.2	4.0	6.7	3.8
180	7.1	7.5	7.2	4.0	6.8	4.0

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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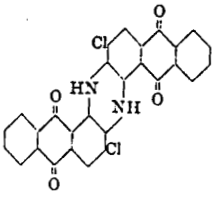
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Vat Blue 6 C. I. No. 69825 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Tamol SN (15%)

   Conditions of storage Aliquot refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-20*

   Avg. wt. 2.5 g; range, 1.2 to 4.5 g. Avg. length 6.5 cm; range, 5.2 to 7.8 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

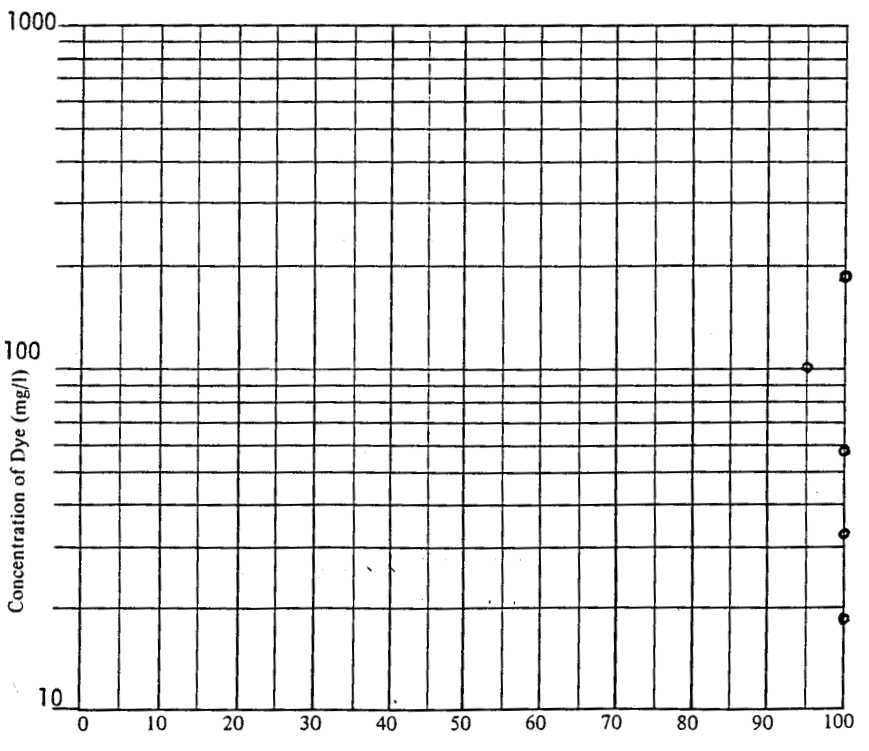
   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 13.1 to 16.5 C. Initial dissolved oxygen 7.2-7.6 mg/l; range during test, 1.9 to 7.6 mg/l. Initial pH 7.1-7.5 ; range during test, 6.6 to 7.5 .

7. 96-hour TL_{50}: >130 mg/l.

*1 jar accidentally stocked twice. (Aerated 5 min each at 48 hr)



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	20	20	20	20	19
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.2	7.4	-	2.7	6.9	1.9
32	7.2	7.5	-	3.1	7.0	2.7
56	7.4	7.4	-	3.3	7.0	3.0
100	7.4	7.4	-	3.5	7.0	2.5
180	7.5	7.2	-	3.2	7.0	2.1

Observations on behavior of fish during the tests: Faint blue at concentrations of 56 mg/l and higher.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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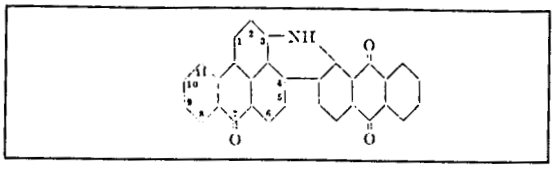
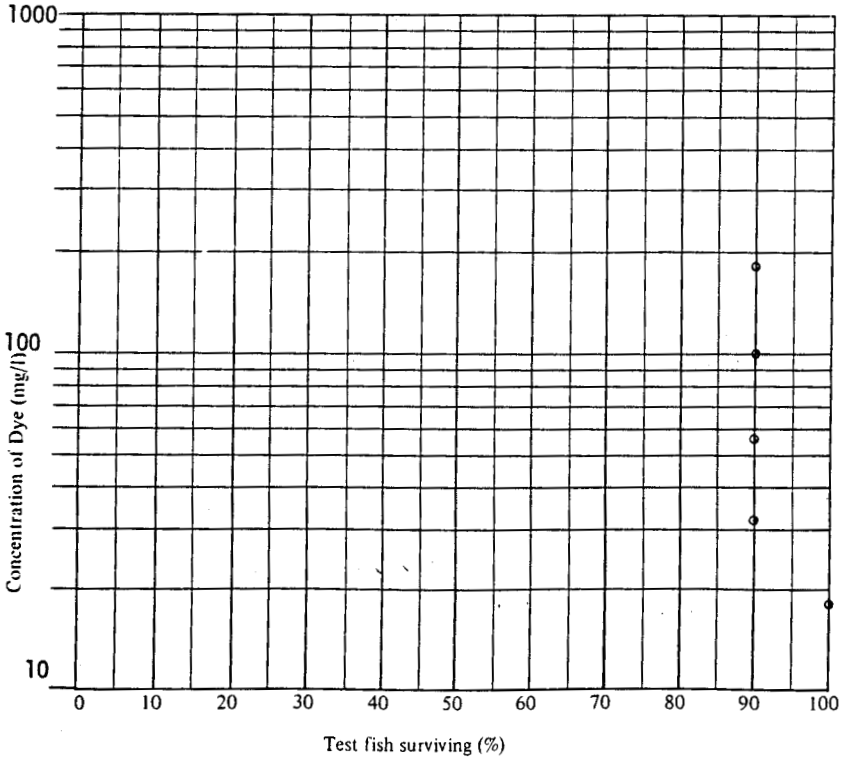
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Vat Green 3 C. I. No. 69500 Date tested 3/23-3/27/72
   Supplier American Dye Manufacturers Inst. Form supplied Composite dispersion of 15% (wt) dye in 15% (wt) Tamol SN
   Conditions of storage Refrigerated
2. Test fish Fathead minnow (Pimephales promelas)
   Supplier Windmill Fish Hatcheries Number per jar 10
   Avg. wt. 0.53 g; range, 0.30 to 0.95 g. Avg. length 4.06 cm; range, 3.30 to 4.80 cm.
3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).
4. Dilution water Charcoal-sand filtered Chapel Hill tap water
   Volume per jar 15 l; depth in jar 30 cm; pH 7.3 ; TOC 5 mg/l; Fe <0.02 mg/l; Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l; turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.
5. Procedure: Static
6. Test conditions: Temperature 15 C; range during test, 15.0 to 16.3 C. Initial dissolved oxygen 6.8-7.6 mg/l; range during test, 5.2 to 7.6 mg/l. Initial pH 7.2-8.4 ; range during test, 6.6 to 8.4 .
7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	-	-	-	9
56	10	-	-	-	9
100	10	-	-	-	9
180	10	-	-	-	9

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.2	7.6			6.8	5.2
32	7.2	7.2			6.6	5.2
56	7.3	7.0			6.7	5.5
100	7.8	7.0			6.7	5.5
180	8.4	6.9			6.8	5.6

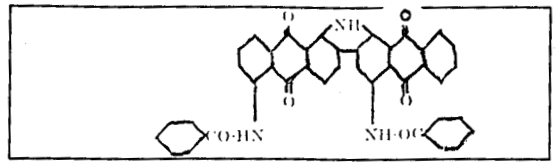
Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

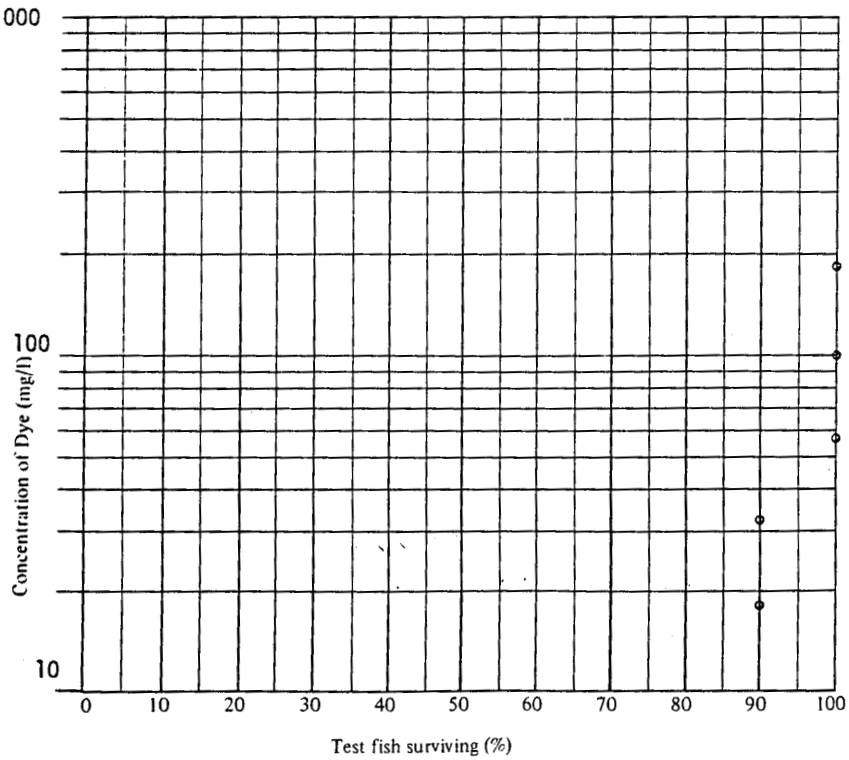
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Vat Brown 3 C. I. No. 69015 Date tested 3/23-3/27/72
   Supplier American Dye Manufacturers Inst. Form supplied Composite dispersion of 15% (wt)/ dye in 15% (wt) Tamol SN
   Conditions of storage Refrigerated
2. Test fish Fathead minnow (Pimephales promelas)
   Supplier Windmill Fish Hatcheries Number per jar 10
   Avg. wt. 0.66 g; range, 0.32 to 1.36 g. Avg. length 4.21 cm; range, 3.40 to 5.40 cm.
3. Test jars: Material glass ; capacity 30 l; dimensions 25 cm(d) x 47 cm(h).
4. Dilution water Charcoal-sand filtered Chapel Hill tap water
   Volume per jar 15 l; depth in jar 30 cm; pH 7.3 ; TOC 5 mg/l; Fe <0.02 mg/l;
   Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l;
   turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.
5. Procedure: Static
6. Test conditions: Temperature 15 C; range during test, 15.1 to 17.0 C. Initial dissolved oxygen 6.8-7.3 mg/l; range during test, 5.2 to 7.3 mg/l. Initial pH 7.1-7.2 ; range during test, 6.6 to 7.2 .
7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	-	-	-	9
32	10	-	-	-	9
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.2	7.2			6.7	5.4
32	7.2	7.0			6.7	5.5
56	7.2	7.1			6.6	5.3
100	7.1	7.2			6.7	5.2
180	7.1	7.3			6.7	5.2

Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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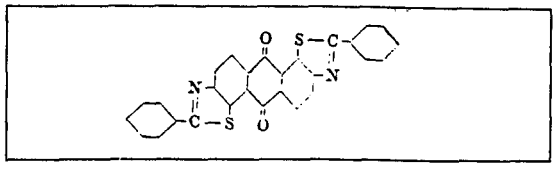
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Vat Yellow 2 C. I. No. 67300 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Tamol SN (15%)

   Conditions of storage Aliquot refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.2 g; range, 1.2 to 4.6 g. Avg. length 6.4 cm; range, 5.2 to 7.9 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

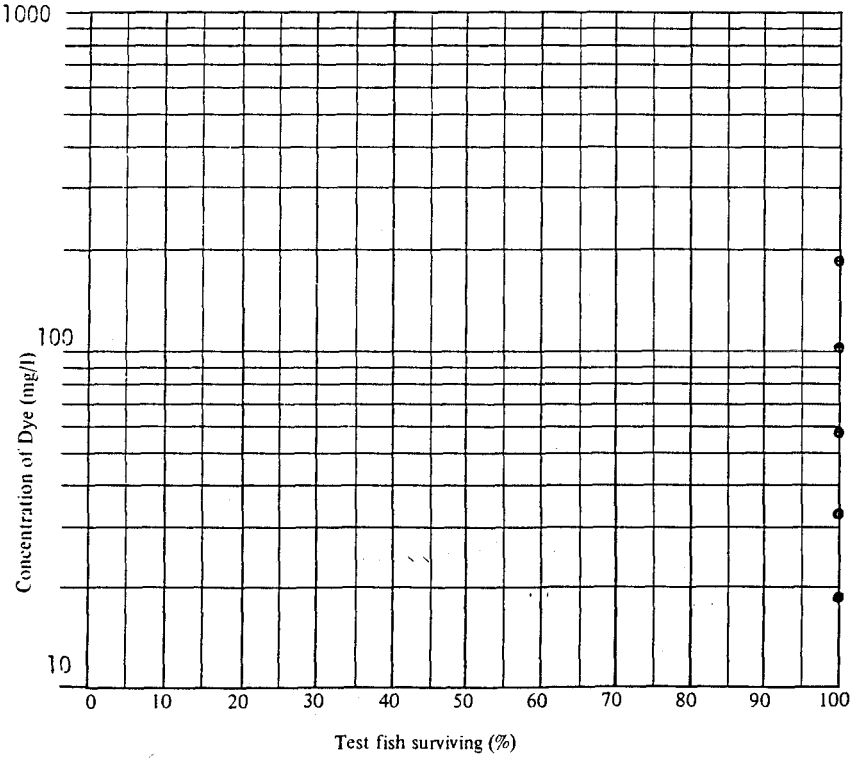
   Al <.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 102 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.2 to 17.0 C. Initial dissolved oxygen 7.5-7.8 mg/l; range during test, 2.0 to 7.8 mg/l. Initial pH 7.1-7.5 ; range during test, 6.6 to 7.5 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}. >180 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

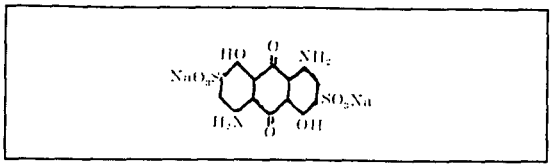
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.3	7.5	-	2.6	6.9	2.0
32	7.4	7.4	-	3.1	6.9	2.5
56	7.5	7.7	-	3.0	7.0	2.4
100	7.5	7.8	-	3.5	7.1	3.0
180	7.5	7.5	-	3.2	6.9	2.4

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

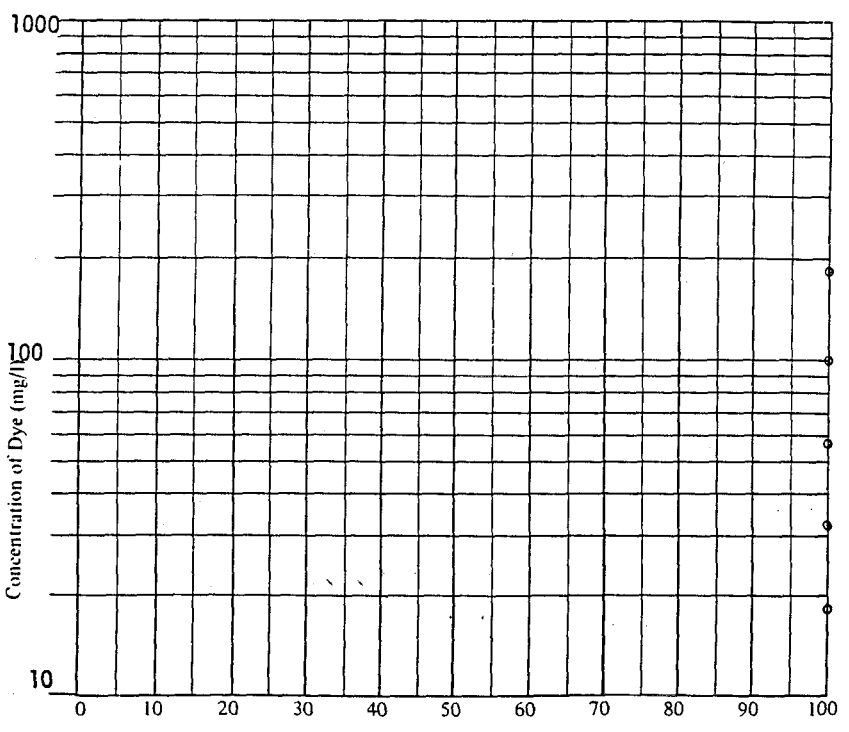
Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Acid Blue 45 C. I. No. 63010 Date tested 3/6-3/10/72
   Supplier American Dye Manufacturers Inst. Form supplied Dry powder
   Conditions of storage Stored in dark at approximately 20 C.
2. Test fish Fathead minnow (Pimephales promelas)
   Supplier Windmill Fish Hatcheries Number per jar 10
   Avg. wt. 0.67 g; range, 0.28 to 2.94 g. Avg. length 4.33 cm; range, 3.50 to 7.00 cm.
3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).
4. Dilution water charcoal-sand filtered Chapel Hill tap water
   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;
   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 5.5 mg/l; total dissolved solids 59 mg/l;
   turbidity 0 JTU; total alkalinity 15 mg/l as $CaCO_3$.
5. Procedure: Static
6. Test conditions: Temperature 15 C; range during test, 13.2 to 17.2 C. Initial dissolved oxygen 6.9-7.5 mg/l; range during test, 5.6 to 7.5 mg/l. Initial pH 6.9-8.7 ; range during test, 6.7 to 8.7 .
7. 96-hour TL_{50}: >180 mg/l. (Aerated 5 min each at 48 hr)



Acclimation of fish: Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C

Pretreatment of fish: Treated with tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.92	7.5	6.81	6.2	6.70	6.6
18	7.41	7.1	7.15	6.2	6.79	6.2
32	7.92	7.0	7.13	6.2	6.80	5.6
56	8.12	7.0	7.23	6.1	6.90	6.3
100	8.53	6.9	7.51	6.4	7.10	6.3
180	8.72	7.1	7.75	6.4	7.20	5.8

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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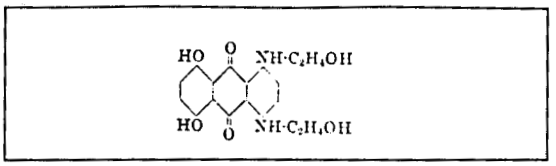
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Disperse Blue 7 C. I. No. 62500 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt.) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in glass container.

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.5 g; range, 1.2 to 4.7 g. Avg. length 6.5 cm; range, 5.2 to 8.0 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

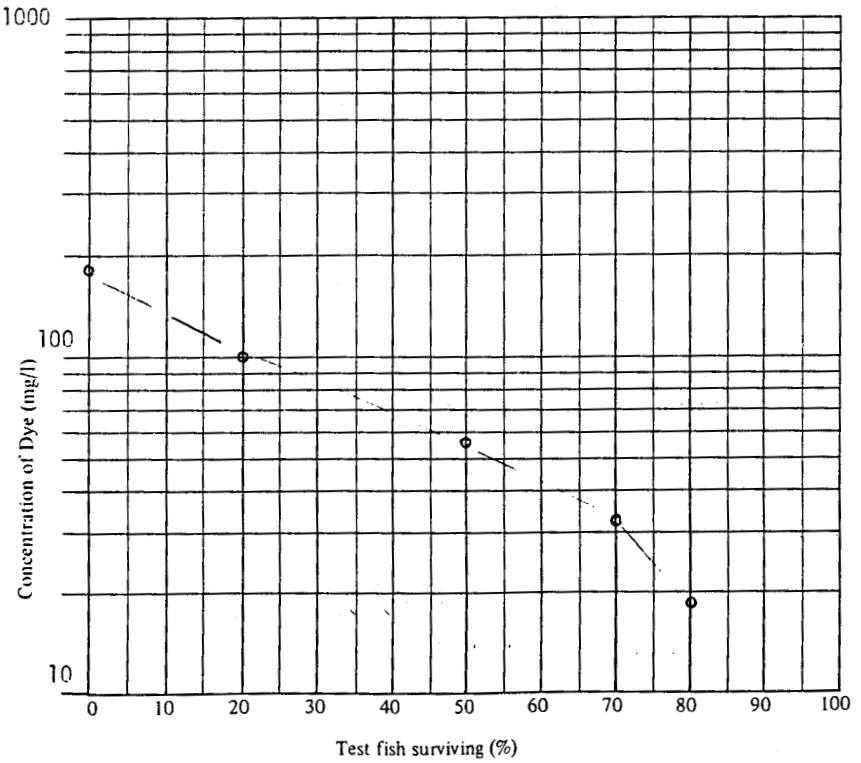
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.1 to 17.2 C. Initial

   dissolved oxygen 7.5-7.6 mg/l; range during test, 2.5 to 7.6 mg/l. Initial pH 7.1-7.4 ;

   range during test, 6.6 to 7.3 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: 52 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	9	9	8
32	10	10	9	8	7
56	10	8	8	7	5
100	10	10	8	5	2
180	10	7	3	2	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.2	7.5	-	3.7	6.8	3.1
32	7.3	7.6	-	4.5	6.8	4.2
56	7.3	7.5	-	4.0	6.8	4.0
100	7.4	7.5	-	4.2	7.0	4.4
180	7.6	7.6	-	3.2	7.0	2.5

Observations on behavior of fish during the tests: Fish were dyed blue.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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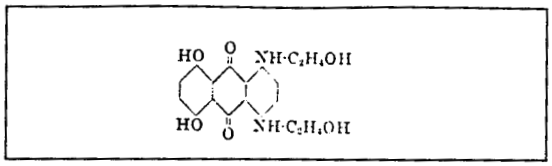


Dye formula

1. Test dye Disperse Blue 7 C. I. No. 62500 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt.) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in glass container.

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.5 g; range, 1.2 to 4.7 g. Avg. length 6.5 cm; range, 5.2 to 8.0 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

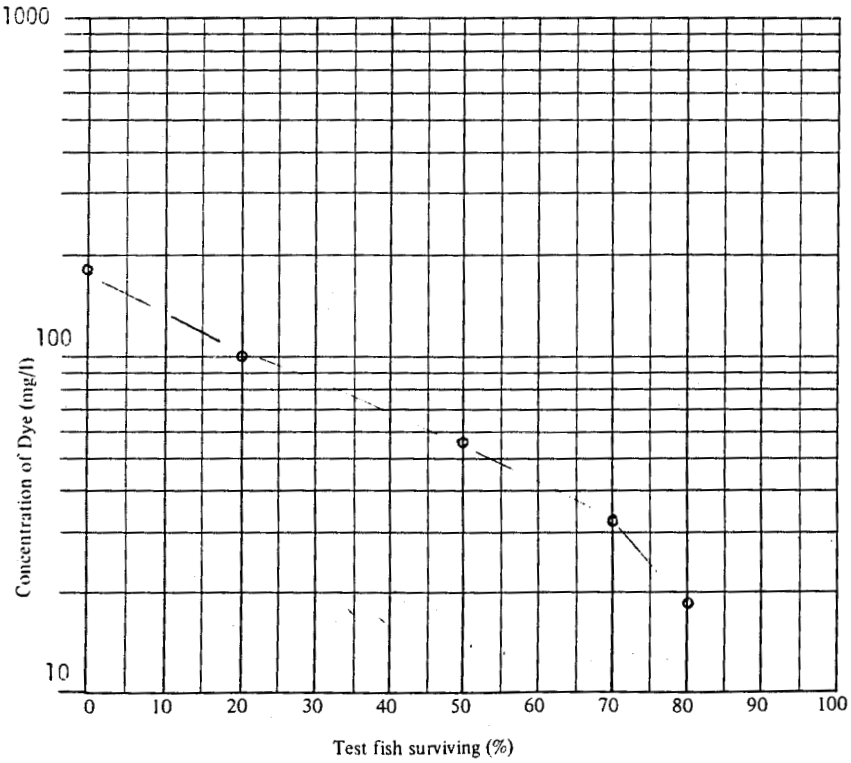
4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6; TOC 6 mg/l; Fe <0.02 mg/l; Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l; turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.1 to 17.2 C. Initial dissolved oxygen 7.5-7.6 mg/l; range during test, 2.5 to 7.6 mg/l. Initial pH 7.1-7.4; range during test, 6.6 to 7.3. (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: 52 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
10	10	7	7	7	7
18	10	2	2	2	1*
32	10	0	-	-	-
56	10	0	-	-	-
100	10	0	-	-	-

* Sick at termination of test.

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.9	7.0	6.2	2.7	6.5	2.6
10	7.2	7.5	6.4	3.2	6.5	2.9
18	7.2	7.4	6.2	1.2	6.7	2.1
32	7.2	7.3	6.9	6.6	-	-
56	7.2	7.4	6.9	6.5	-	-
100	7.2	7.3	6.9	6.7	-	-

Observations on behavior of fish during the tests: All dead fish dyed. Surviving fish dyed blue-green.

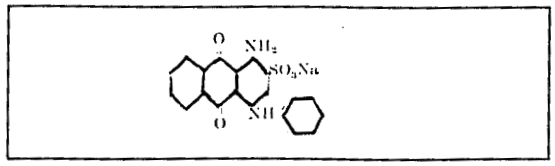
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Acid Blue 25 C. I. No. 62055 Date tested 1/31/72

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Stored at c 20 C in dark

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 2.6 g; range, 0.90 to 5.31 g. Avg. length 6.6 cm; range, 4.80 to 8.20 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.9 ; TOC 2 mg/l; Fe <0.2 mg/l;

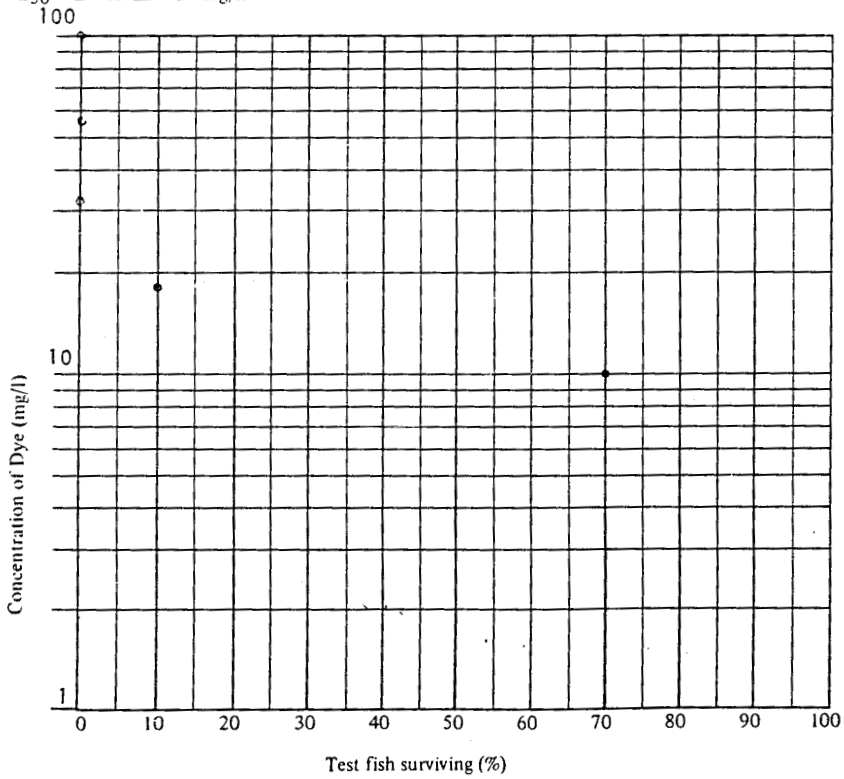
   Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l;

   turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.8 to 17.0 C. Initial dissolved oxygen 7.0-7.5 mg/l; range during test, 1.2 to 7.5 mg/l. Initial pH 6.9-7.2 ; range during test, 6.2 to 7.2 . (Aerated for 5 min each at 48 hr)

7. 96-hour TL_{50}: 12 mg/l.



Acclimation of fish Fish received October 20, 1971. Maintained in 250 gal. plastic tank receiving constant flow of filtered tap water and aerated with compressed air. Temp. 15-20 C.

Pretreatment of fish Treated on October 20-22, Nov. 1, and Nov. 16-18, 1971 with Tetrachel (Tetracycline H Cl) to control disease. Treated with Na Cl 11/1. Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	9	9	9
1.0	10	10	10	10	10
1.8	10	10	10	9	9
3.2	10	10	10	8	8
5.6	10	10	9	8	8
10.0	10	6	1	1	1

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.0	6.6	4.4	6.4	3.6
1.0	7.1	6.9	6.5	4.7	6.5	4.5
1.8	7.2	6.9	6.5	5.1	6.5	4.0
3.2	7.2	7.1	6.5	5.2	6.5	3.9
5.6	7.2	7.0	6.5	5.0	6.5	3.8
10.0	7.2	6.9	6.5	4.8	6.6	4.7

Observations on behavior of fish during the tests: Before death, fish tended to become very slow-moving at higher concentrations. Dead fish were dyed on heads and fins. Surviving fish were dyed dark green in concentrations above 1 mg/l.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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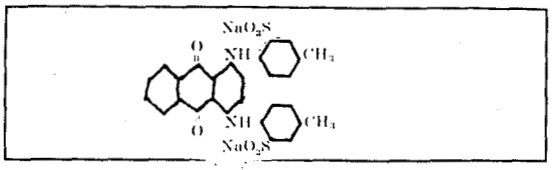
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Acid Green 25 C. I. No. 61570 Date tested 11/29-12/3/71

   Supplier ADMI Form supplied Powder - composite

   Conditions of storage Stored dry at c.20 C in dark; stock solutions refrigerated in glass

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.2 g; range, 0.4 to 2.9 g. Avg. length 5.2 cm; range, 3.5 to 7.5 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 3 mg/l; Fe <0.01 mg/l;

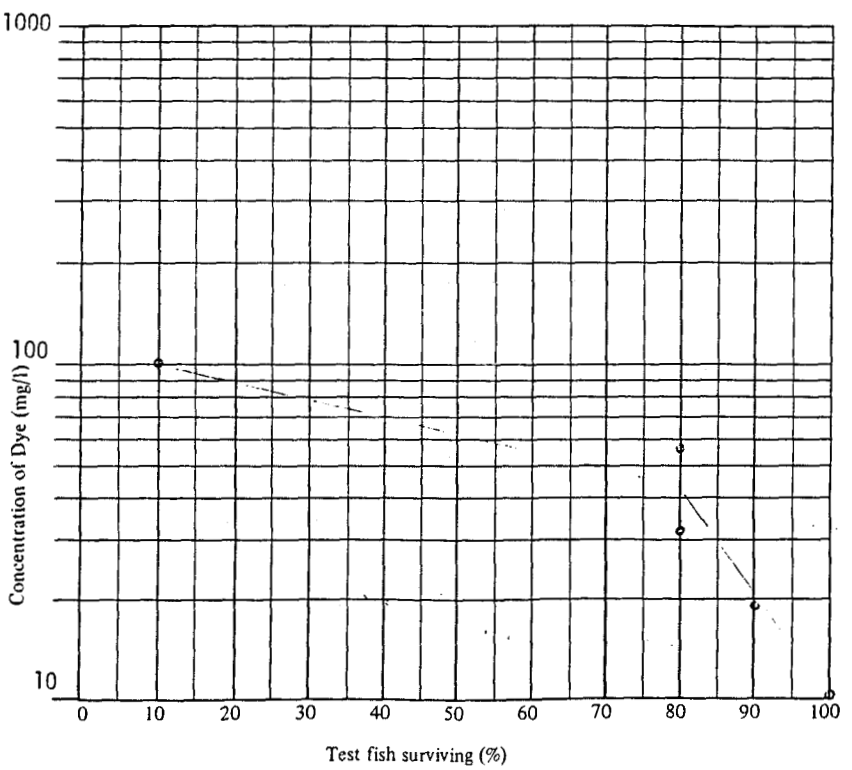
   Al <0.1 mg/l; Mg 0.53 mg/l; Ca 8.6 mg/l; total dissolved solids 121 mg/l;

   turbidity 0 JTU; total alkalinity 29 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 18 C; range during test, 17.9 to 20 C. Initial dissolved oxygen 6.9-7.1 mg/l; range during test, 3.6 to 7.1 mg/l. Initial pH 7.1-7.2 ; range during test, 6.4 to 7.2 . (Aerated for 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 6.2 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline HCl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
0.10	10	10	10	10	10
0.18	10	10	10	10	10
0.32	10	10	10	10	10
0.56	10	10	10	10	10
1.00	10	10	10	10	7*

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
0.10	7.2	7.6	-	3.8	6.6	2.7
0.18	7.3	7.5	-	3.5	6.6	2.5
0.32	7.3	7.6	-	2.9	6.6	2.6
0.56	7.2	7.3	-	2.5	6.7	2.3
1.00	7.3	7.5	-	2.4	6.7	1.7

*Observations on behavior of fish during the tests: At 96 hr at 1.00 mg/l, two additional fish were showing severe distress and death appeared imminent. Respiratory distress was not observed.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

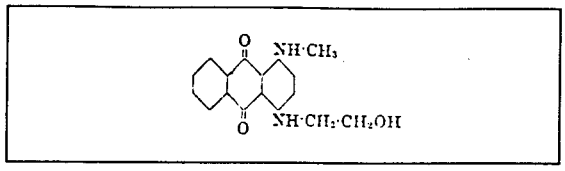
Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER RESEARCH CENTER

FISH BIOASSAY TESTS

AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Disperse Blue 3 C. I. No. 61505 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in plastic stock bottle.

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.5 g; range, 1.1 to 4.6 g. Avg. length 6.6 cm; range, 5.2 to 8.0 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

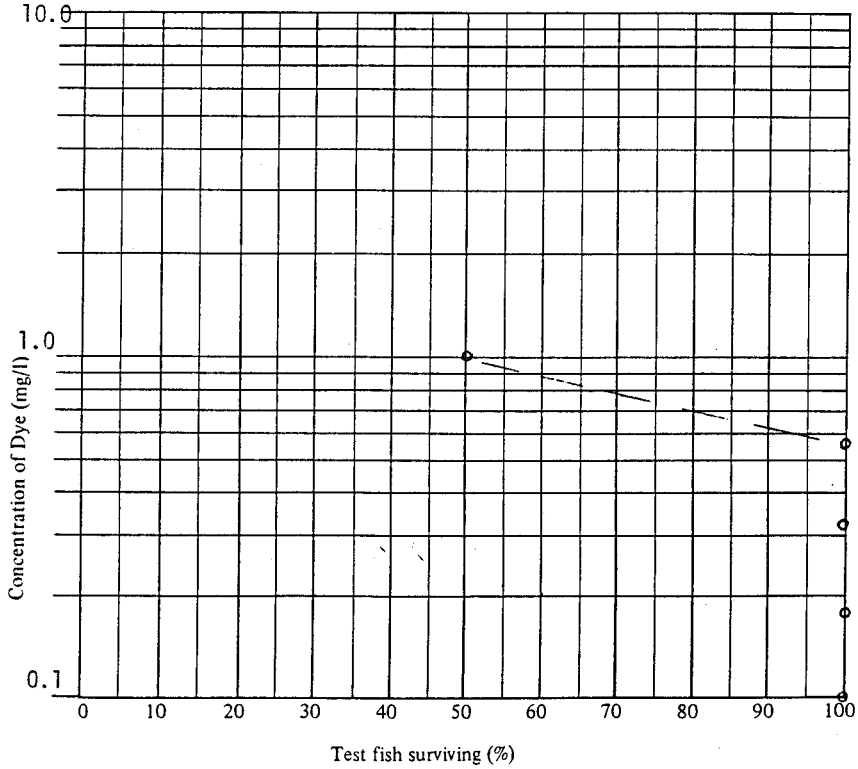
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.4 to 17.2 C. Initial

   dissolved oxygen 7.3-7.6 mg/l; range during test, 1.7 to 7.6 mg/l. Initial pH 7.1-7.3 ;

   range during test, 6.6 to 7.3 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: 1 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	-	-	-	7

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.1	7.0			6.7	5.3
32	7.2	7.1			6.6	5.5
56	7.2	7.1			6.6	5.2
100	7.2	7.2			6.7	5.4
180	7.4	7.2			6.8	5.3

Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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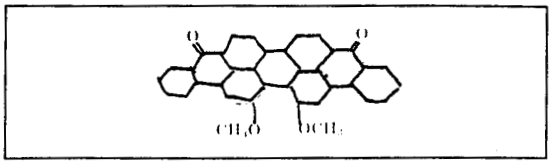
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Vat Green 1 C. I. No. 59825 Date tested 3/23-3/27/72

   Supplier American Dye Manufacturers Inst Form supplied Composite dispersion of 15% (wt) dye in 15% (wt) Tamol SN

   Conditions of storage Refrigerated

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.60 g; range, 0.25 to 0.97 g. Avg. length 4.12 cm; range, 3.20 to 5.00 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

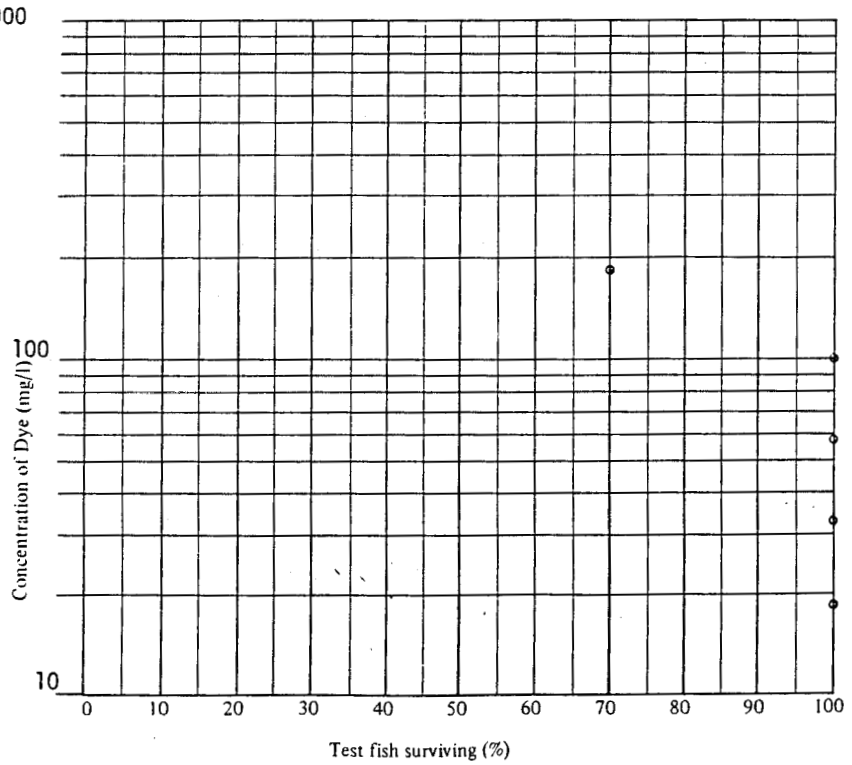
4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.3 ; TOC 5 mg/l; Fe <0.02 mg/l; Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l; turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.0 to 16.6 C. Initial dissolved oxygen 6.8-7.2 mg/l; range during test, 5.2 to 7.2 mg/l. Initial pH 7.1-7.4 ; range during test, 6.6 to 7.4 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.3	7.7	-	3.0	6.9	2.1
32	7.4	7.6	-	3.0	6.9	1.7
56	7.4	7.7	-	2.8	7.0	1.5
100	7.5	7.5	-	2.5	7.0	1.8
180	7.5	7.6	-	2.9	7.0	2.0

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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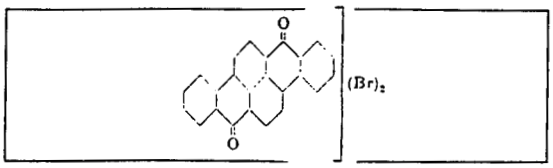
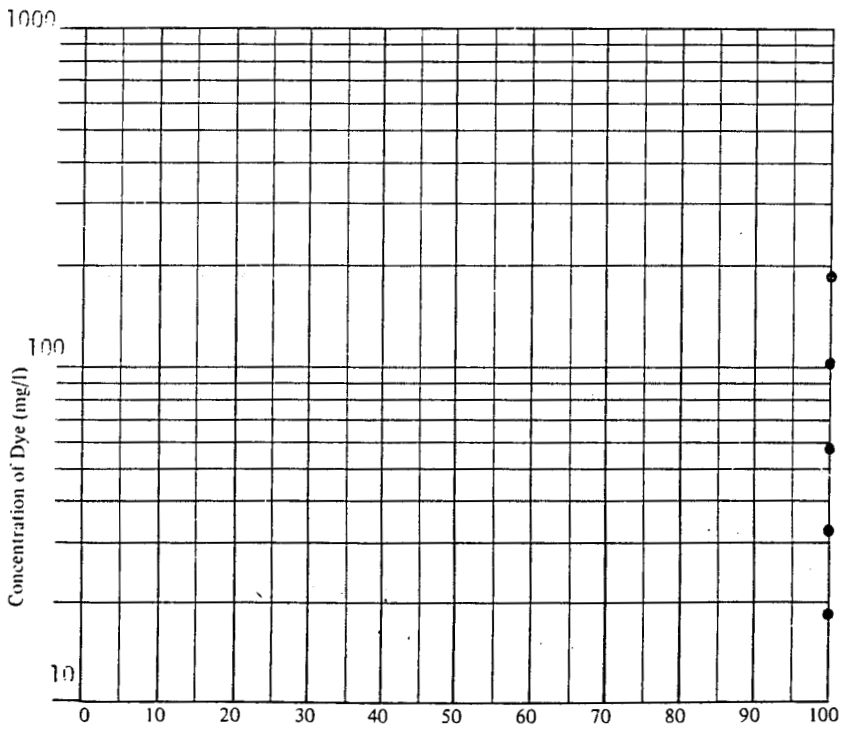


Dye formula

1. Test dye Vat Orange 1 C. I. No. 59105 Date tested Jan. 6-10, 1972
   Supplier ADMI Form supplied Dispersion of 15% (wt) with Tamol SN (15
   Conditions of storage Aliquot refrigerated in glass container
2. Test fish Fathead minnow (Pimephales promelas)
   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11
   Avg. wt. 2.3 g; range, 0.6 to 4.4 g. Avg. length 6.4 cm; range, 4.5 to 7.9 cm.
3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).
4. Dilution water charcoal and sand filtered Chapel Hill tap water
   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;
   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;
   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.
5. Procedure: Static
6. Test conditions: Temperature 15 C; range during test, 14.0 to 17.2 C. Initial
   dissolved oxygen 7.6-7.7 mg/l; range during test, 1.5 to 7.7 mg/l. Initial pH 7.1-7.5 ;
   range during test, 6.6 to 7.5 .
7. 96-hour TL_{50}: >100 mg/l.



Acclimation of fish: Fish received 2/14/72; maintained in constantly flowing, a
charcoal-sand filtered Chapel Hill tap water 14-18 C

Pretreatment of fish: Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving		
		24 hr.	48 hr.	72 hr.
0	10	10	10	10
18	10	10	10	10
32	10	10	10	10
56	10	10	10	10
100	10	10	10	10
180	10	-	-	-

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/
0	7.2	6.8			6.6	5.2
18	7.2	7.1			6.6	5.4
32	7.2	6.9			6.5	5.4
56	7.2	7.3			6.6	5.0
100	7.2	7.0			6.5	5.2
180	7.2	6.8			6.4	4.2

Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Wat* 13th ed., APHA, AWWA, WPCF, New York, N.Y. (1971). The tests are subject therefore to the applications and li procedures. Fish tests and their application are described in *Standard Methods* as follows:

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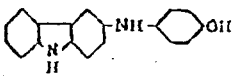
UNC WASTEWATER RESEARCH CENTER | FISH BIOASSAY TESTS

AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT

sulfide condensation product from



3-Carbazolylaminophenol

Dye formula

1. Test dye Vat BTue 43 C. I. No. 53630 Date tested 3/23-3/27/72

Supplier American Dye Manufacturers Inst Form supplied Composite dispersion of 15% (wt) dye in 15% (wt) Tamol SN

Conditions of storage Refrigerated

2. Test fish Fathead minnow (Pimephales promelas)

Supplier Windmill Fish Hatcheries Number per jar 10

Avg. wt. 0.62 g; range, 0.33 to 1.25 g. Avg. length 4.23 cm; range, 3.70 to 5.00 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

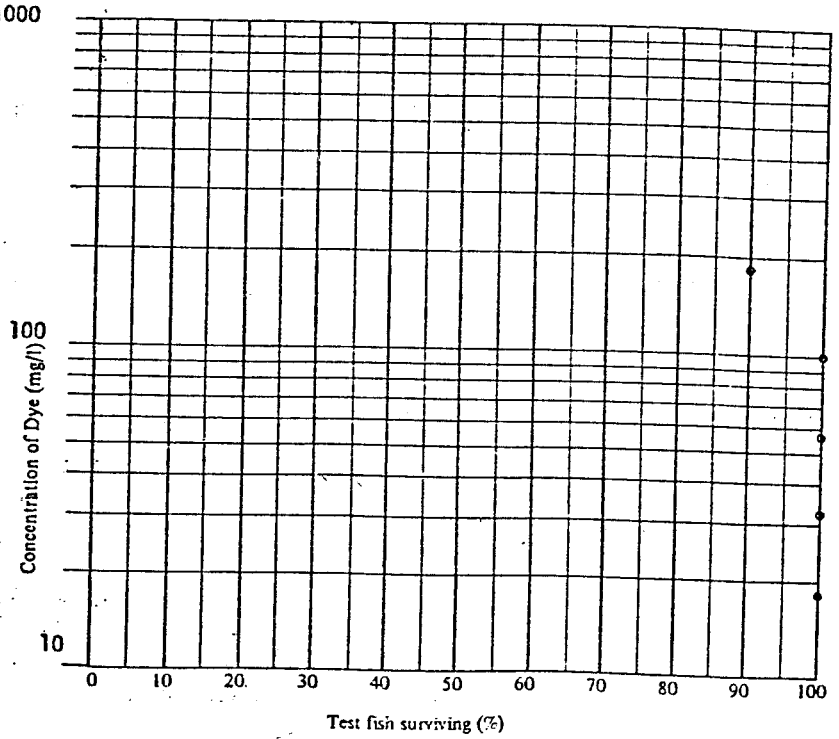
4. Dilution water Charcoal-sand filtered Chapel Hill tap water

Volume per jar 15 l; depth in jar 30 cm; pH 7.3; TOC 5 mg/l; Fe <0.02 mg/l; Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l; turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.0 to 16.3 C. Initial dissolved oxygen 6.8-7.3 mg/l; range during test, 4.2 to 7.3 mg/l. Initial pH 7.2; range during test, 6.4 to 7.2.

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerat

charcoal-sand filtered Chapel hill tap water 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/7

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 h
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.1	6.5			6.7	4.5
32	7.1	6.3			6.6	5.0
56	7.1	6.4			6.6	5.1
100	7.1	6.5			6.7	5.0
180	7.1	6.4			6.7	4.8

Observations on behavior of fish during the tests: Not remarkable.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Was* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, de permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate info on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measure relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent co tions that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT

sulfide condensation product from

2,4-Dinitrophenol

Dye formula

1. Test dye Sulfur Black 1 C. I. No. 53185 Date tested 3/23-3/27/72

Supplier American Dye Manufacturers Inst Form supplied Composite dispersion of 15% (wt) dye in 15% (wt) Tamol SN

Conditions of storage Refrigerated

2. Test fish Fathead minnow (Pimephales promelas)

Supplier Windmill Fish Hatcheries Number per jar 10

Avg. wt. 0.66 g; range, 0.30 to 1.41 g. Avg. length 4.34 cm; range, 3.50 to 5.20 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

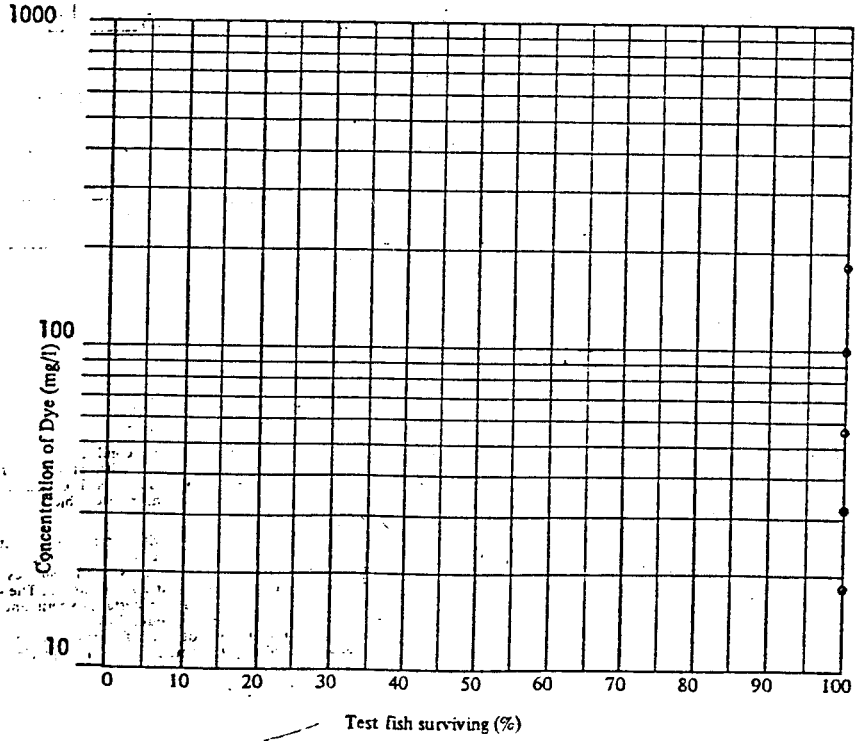
4. Dilution water charcoal-sand filtered Chapel Hill tap water

Volume per jar 15 l; depth in jar 30 cm; pH 7.3 ; TOC 5 mg/l; Fe <0.02 mg/l; Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l; turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 14.3 to 16.0 C. Initial dissolved oxygen 6.3-6.8 mg/l; range during test, 4.2 to 6.8 mg/l. Initial pH 7.1-7.2 ; range during test, 6.6 to 7.2 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
1.8	10	10	10	10	10
3.2	10	-	-	-	9
5.6	10	-	-	7	3
10.0	10	6	-	0	0
18.0	10	1	0	0	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.18	6.8	-	-	6.55	5.2
1.8	7.00	6.2	-	-	6.30	5.6
3.2	7.05	6.2	-	-	6.58	5.3
5.6	7.00	6.4	-	-	6.54	5.3
10.0	7.05	6.4	-	-	6.56	4.7
18.0	7.00	6.6	6.89	5.9	-	-

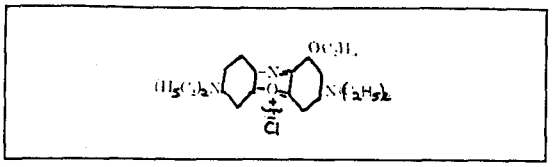
Observations on behavior of fish during the tests: Dead fish dyed pale blue.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of thes procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determin permissible effluent discharge rates; establish the relative sensitivity of various fish species, and identify effects of physical and chemica variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by wate pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxican under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate informatio on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurat estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in wate with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of th relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentra tions that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and stil lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Basic Blue 3 C. I. No. 51005 Date tested 3/23-3/27/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.60 g; range, 0.32 to 1.35 g. Avg. length 4.13 cm; range, 3.20 to 5.70 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.3 ; TOC 5 mg/l; Fe <0.02 mg/l;

   Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l;

   turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

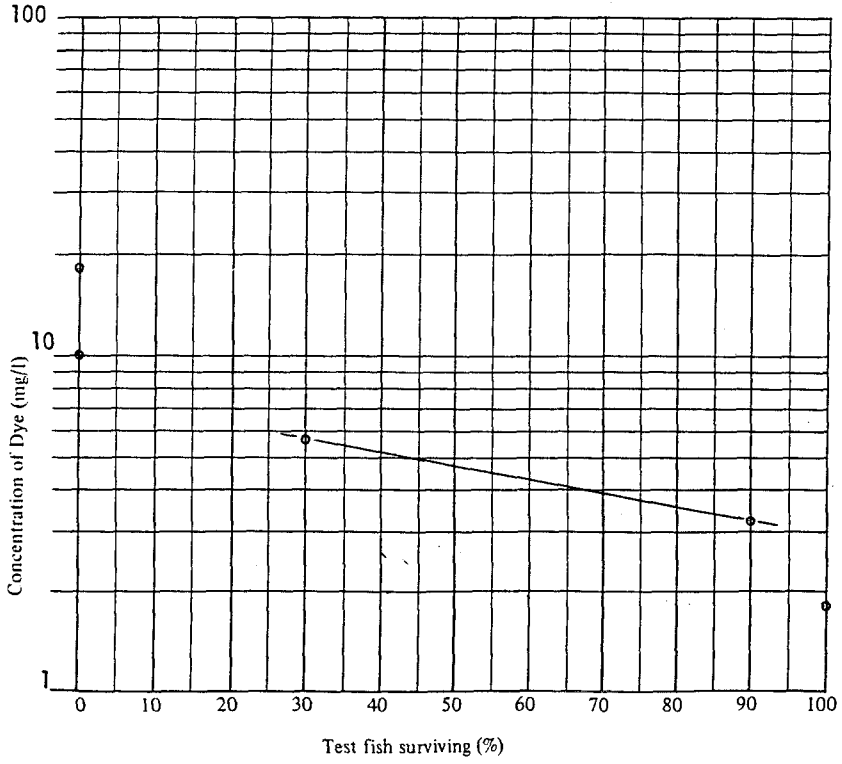
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 15.1 to 17.7 C. Initial

   dissolved oxygen 6.2-6.8 mg/l; range during test, 4.7 to 6.8 mg/l. Initial pH 7.0-7.2 ;

   range during test, 6.3 to 7.2 .

7. 96-hour TL_{50}: 4 mg/l.



Acclimation of fish Fish received Oct. 20, 1971. Maintained in 250 gal. plastic tank receiving constant flow of filtered tap water and aerated with compressed air. Temp. 15-20 C.

Pretreatment of fish Treated on Oct. 20-22, Nov. 1, and Nov. 16-18, 1971 with Tetrachel (tetracycline H Cl) to control disease. Treated with Na Cl 11/1. Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	9	9	9
1.0	10	10	10	10	10
1.8	10	10	9	9	9
3.2	10	9	8	7	5
5.6	10	2	1	0	0
10.0	10	0	0	0	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.0	6.6	4.4	6.4	3.6
1.0	7.0	7.1	6.5	4.7	6.6	4.5
1.8	7.1	7.3	6.4	3.6	6.5	3.9
3.2	7.2	7.4	6.4	4.4	6.6	3.5
5.6	7.1	7.2	6.5	5.0	-	-
10.0	7.2	7.2	7.1	7.2	-	-

Observations on behavior of fish during the tests: Fish dyed faint yellow. At concentrations of 3.2 mg/l and above, fish showed disturbed equilibrium -- swam upside down, on side, etc.

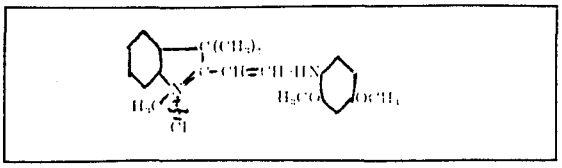
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Basic Yellow 11 C. I. No. 48055 Date tested 11/29-12/3/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage Stored dry at c. 20 C in dark; stock solutions refrigerated in glass

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.5 g; range, 0.4 to 13.6 g. Avg. length 5.6 cm; range, 3.5 to 7.5 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 3 mg/l; Fe <0.01 mg/l;

   Al <0.1 mg/l; Mg 0.53 mg/l; Ca 8.6 mg/l; total dissolved solids 121 mg/l;

   turbidity 0 JTU; total alkalinity 29 mg/l as $CaCO_3$.

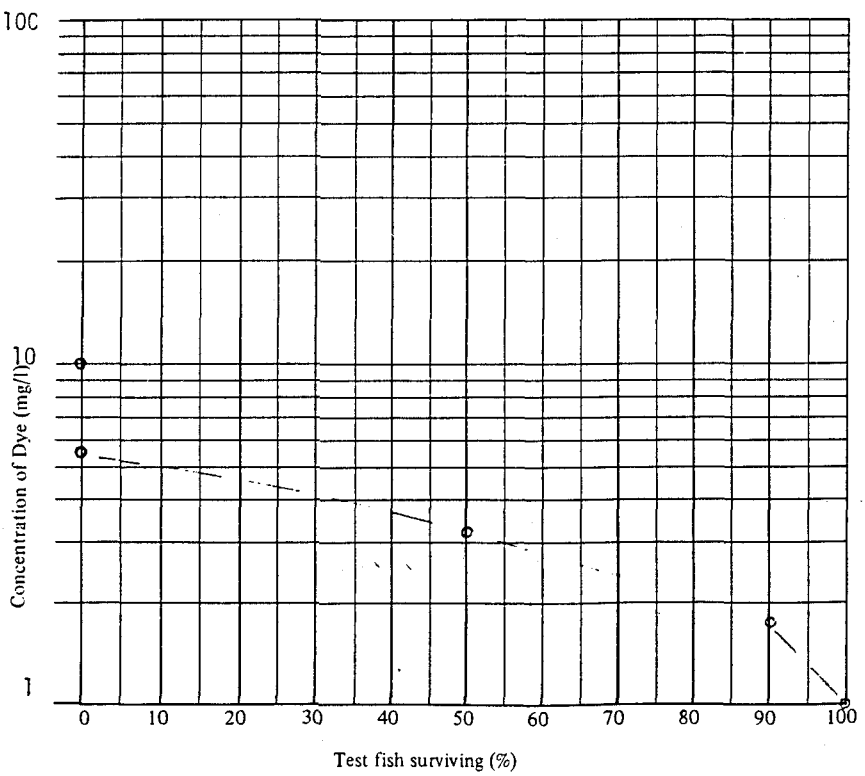
5. Procedure: Static

6. Test conditions: Temperature 18 C; range during test, 17 to 20 C. Initial

   dissolved oxygen 7.0-7.4 mg/l; range during test, 3.5 to 7.4 mg/l. Initial pH 7.0-7.2 ;

   range during test, 6.4 to 7.2 . (Aerated for 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 3.2 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	9
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
18	7.2	7.6	-	2.9	6.9	3.0
32	7.4	7.6	-	2.5	7.0	2.4
56	7.6	7.7	-	2.6	6.9	2.5
100	7.7	7.8	-	2.5	7.0	2.7
180	7.9	7.6	-	1.9	7.0	2.3

Observations on behavior of fish during the tests: Fish dyed faint gold at 56 mg/l and above.

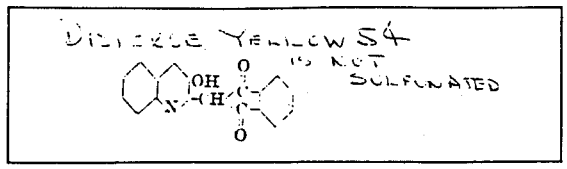
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Disperse Yellow 54 C. I. No. 47020 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dispersion of 15% (wt) with Reax 83-A (15%)

   Conditions of storage Aliquot refrigerated in glass container

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.3 g; range, 1.0 to 5.0 g. Avg. length 6.6 cm; range, 5.2 to 7.8 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6; TOC 6 mg/l; Fe <0.02 mg/l;

   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 100 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

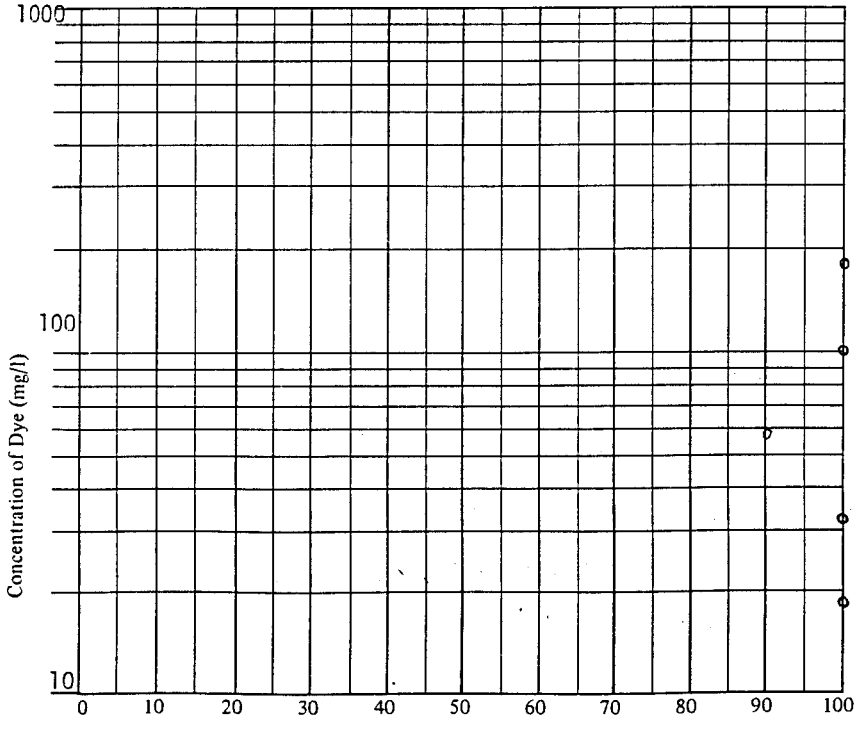
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 13.9 to 17.1 C. Initial

   dissolved oxygen 7.6-7.8 mg/l; range during test, 1.9 to 7.8 mg/l. Initial pH 7.1-7.9;

   range during test, 6.6 to 7.8. (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}: >130 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water, 14.5-18.5 C.

Pretreatment of fish Treated with tetracycline H Cl on 12/13.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	11	11	11	11	11
0.032	10	10	10	10	10
0.056	10	10	7	5	2
0.10	10	10	7	4	2
0.18	10	7	2	0	0
0.32	10	0	0	0	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.6	-	3.0	6.6	3.1
0.032	7.1	7.5	-	2.5	6.8	1.7
0.056	7.2	7.5	-	1.4	6.8	1.5
0.10	7.2	7.3	-	1.6	6.9	1.7
0.18	7.3	7.4	6.9	3.1	-	-
0.32	7.3	7.4	7.0	5.5	-	-

Observations on behavior of fish during the tests: Fish dyed faint purple.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT

A mixture of the hydrochlorides of the more highly methylated pararosanilines, containing principally the N-tetra-, penta-, and hexamethyl derivatives, obtained by oxidation of *N,N*-dimethylaniline with cupric chloride, or by the action of air on an intimate mixture of *N,N*-dimethylaniline, phenol, sodium chloride, and copper sulfate

Dye formula

1. Test dye Basic Violet 1 C. I. No. 42535 Date tested Jan. 6-10, 1972

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Dry powder stored at c.20 C in dark. Stock solution prepared immediately before test

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries, Kernersville Number per jar 10-11

   Avg. wt. 2.7 g; range, 0.8 to 5.0 g. Avg. length 6.6 cm; range, 5.0 to 8.0 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal and sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.6 ; TOC 6 mg/l; Fe <0.02 mg/l;

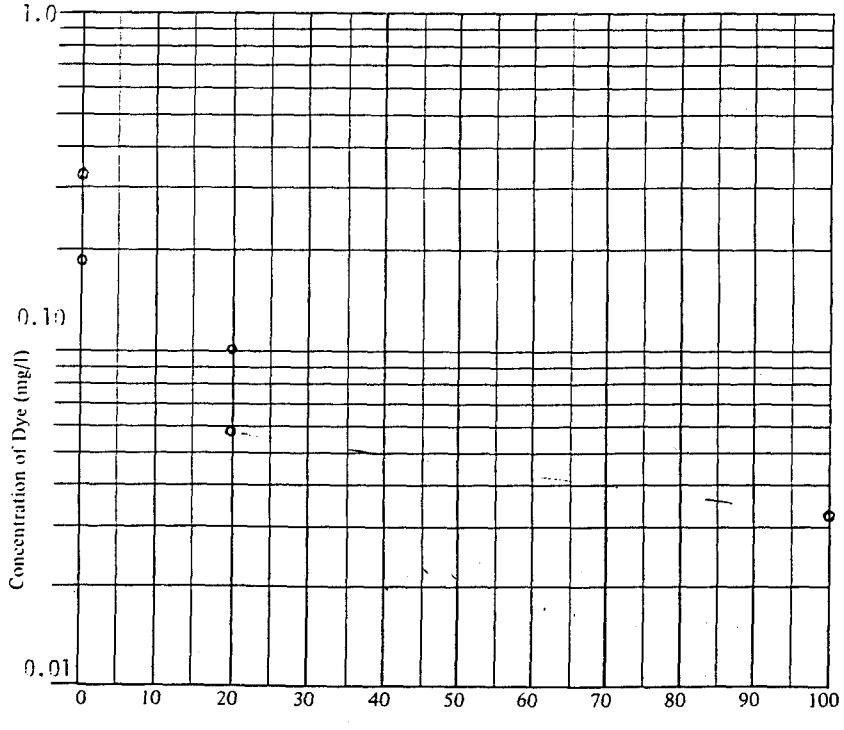
   Al <0.1 mg/l; Mg 1.4 mg/l; Ca 8.5 mg/l; total dissolved solids 108 mg/l;

   turbidity 0 JTU; total alkalinity 27 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 13.2 to 17.2 C. Initial dissolved oxygen 7.3-7.6 mg/l; range during test, 1.4 to 7.6 mg/l. Initial pH 7.1-7.3 ; range during test, 6.6 to 7.3 . (Aerated 5 min each at 48 hr)

7. 96-hour TL_{50}. 0.047 mg/l.



Acclimation of fish Fish received Oct. 20, 1971. Maintained in 250 gal. plastic tar
receiving constant flow of filtered tap water and aerated with compressed ai
Temp. 15-20 C.

Pretreatment of fish Treated on Oct. 20-22, Nov. 1, and Nov. 16-18, 1971 with Tetrach
(Tetracycline H Cl) to control disease. Treated with Na Cl 11/1. Starved f
48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	9	9	9
0.018	10	10	10	10	10
0.032	11	11	11	11	11
0.056	10	10	10	10	10
0.10	10	10	6	6	6
0.18	10	10	2	2	2

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.0	6.6	4.4	6.4	3.6
0.018	7.1	7.0	6.4	4.8	6.5	4.3
0.032	7.2	7.1	6.5	4.8	6.5	5.0
0.056	7.2	7.2	6.4	4.3	6.5	4.4
0.10	7.2	7.0	6.4	4.7	6.6	5.1
0.18	7.3	6.9	6.5	4.9	6.5	3.5

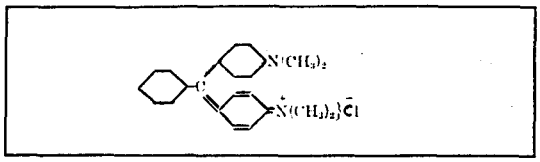
Observations on behavior of fish during the tests: Dead fish not dyed. Some appeared to have
hemorrhages around gills. Fish surviving at concentrations of 0.1 mg/l and
above appeared sluggish.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Waste* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, det permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and ch variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected to under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate inform on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an ac estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent cond tions that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, an lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and g resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Basic Green 4 C. I. No. 42000 Date tested 11/29-12/3/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage Stored dry at c. 20 C in dark; stock solutions refrigerated in glass

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.3 g; range, 0.4 to 2.9 g. Avg. length 5.4 cm; range, 3.5 to 7.5 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1; TOC 3 mg/l; Fe <0.01 mg/l;

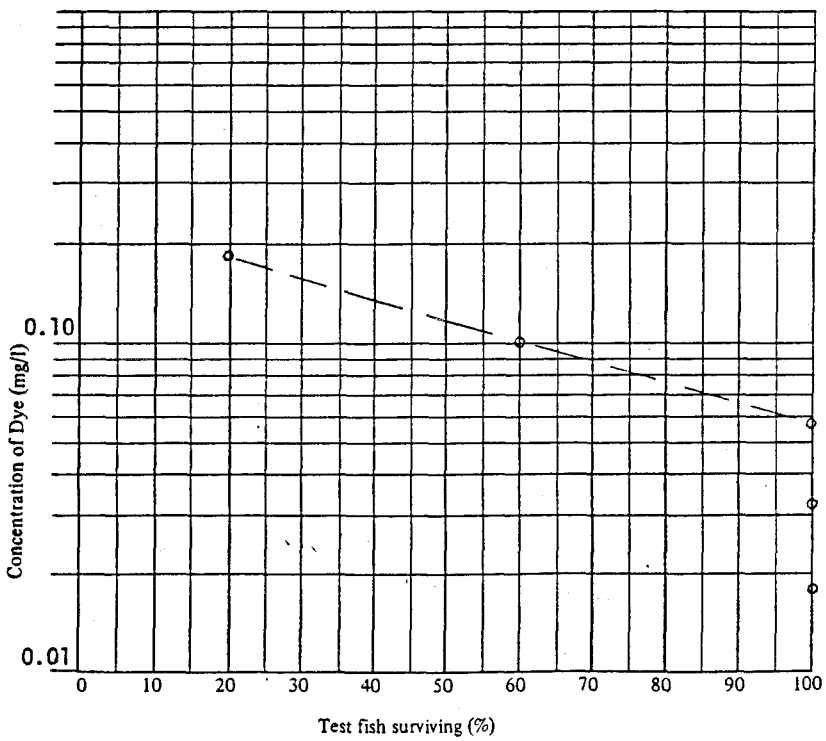
   Al <0.1 mg/l; Mg 0.53 mg/l; Ca 8.6 mg/l; total dissolved solids 121 mg/l;

   turbidity 0 JTU; total alkalinity 29 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 18 C; range during test, 16 to 20 C. Initial dissolved oxygen 6.9-7.2 mg/l; range during test, 3.5 to 7.2 mg/l. Initial pH 7.1-7.3; range during test, 6.4 to 7.3. (Aerated for 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 0.12 mg/l.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in cont
ously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 1l/l; tetracycline
Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.3	7.4	7.3	4.1	6.9	3.9
32	7.2	7.3	7.2	4.0	7.0	4.0
56	7.1	7.4	7.2	4.2	6.8	3.9
100	7.0	7.4	7.2	4.0	6.8	3.8
180	7.0	7.1	7.1	3.8	6.7	3.4

Observations on behavior of fish during the tests: Not remarkable

Note: Dye produced turbidity.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastew* 13th ed., APHA, AWWA, WPCF, New York, N.Y. (1971). The tests are subject therefore to the applications and limitations of t procedures. Fish tests and their application are described in *Standard Methods* as follows:

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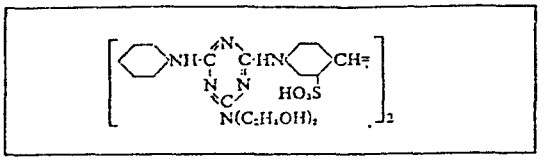
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate inform on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an acc estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures c relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent conce tions that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and gr resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER RESEARCH CENTER | FISH BIOASSAY TESTS

AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Fluorescent Brightening Agent 28 C. I. No. 40622 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solutions refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.3 g; range, 0.5 to 2.6 g. Avg. length 5.1 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material glass; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l, depth in jar 30 cm; pH 7.1; TOC 34 mg/l; Fe 0.63 mg/l;

   Al <0.1 mg/l; Mg 1.0 mg/l, Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU, total alkalinity 32 mg/l as $CaCO_3$.

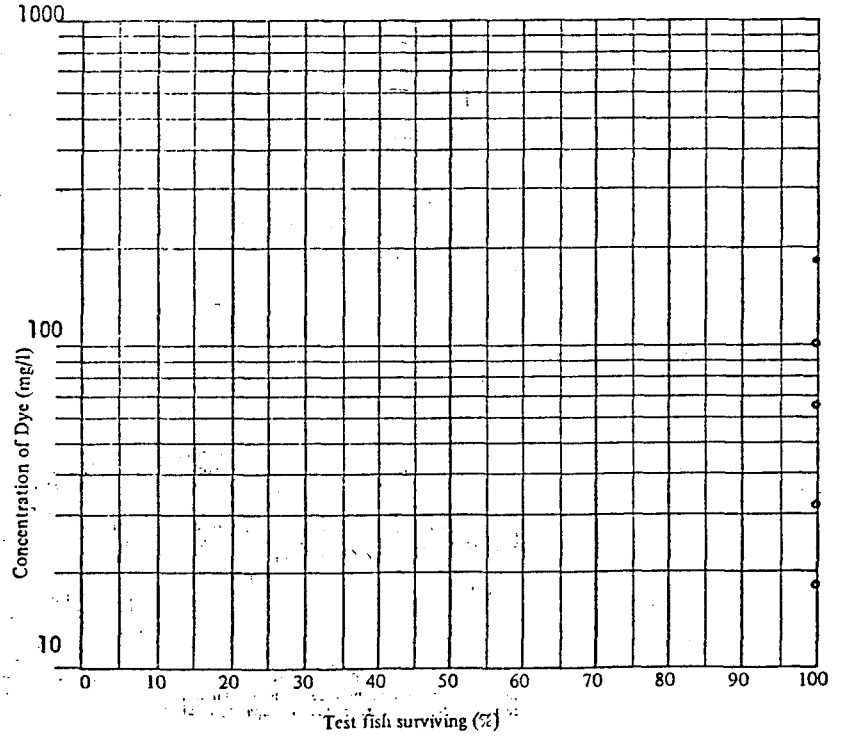
5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.9 to 17.5 C. Initial

   dissolved oxygen 7.1-7.5 mg/l; range during test, 2.8 to 7.5 mg/l. Initial pH 7.0-7.3;

   range during test, 6.7 to 7.5.

7. 96-hour TL_{50}: ≥100 mg/l.



Acclimation of fish Fish received 2/14/72 maintained in constantly flowing, aerated charcoal-sand filtered Chapel Hill tap water 14-18 C.

Pretreatment of fish Treated with tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.4	8.1	6.6	6.9	7.0	6.4
18	6.8	7.3	6.6	5.7	7.2	6.4
32	6.9	7.1	6.8	5.7	7.2	6.1
56	6.9	5.8	6.8	4.3	7.1	6.0
100	7.0	7.4	6.8	5.0	7.1	6.0
180	7.0	7.4	6.8	5.3	7.1	5.8

Observations on behavior of fish during the tests: Not remarkable.

Other observations: In all concentrations of dye there was substantial turbidity which persisted throughout the test.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT

A self-condensation product of 5-nitro-o-toluenesulfonic acid

Dye formula

1. Test dye Direct Yellow 11 C. I. No. 40000 Date tested 3/2-3/6/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead Minnows (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.56 g; range, 0.30 to 0.88 g. Avg. length 4.25 cm; range, 3.40 to 4.90 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

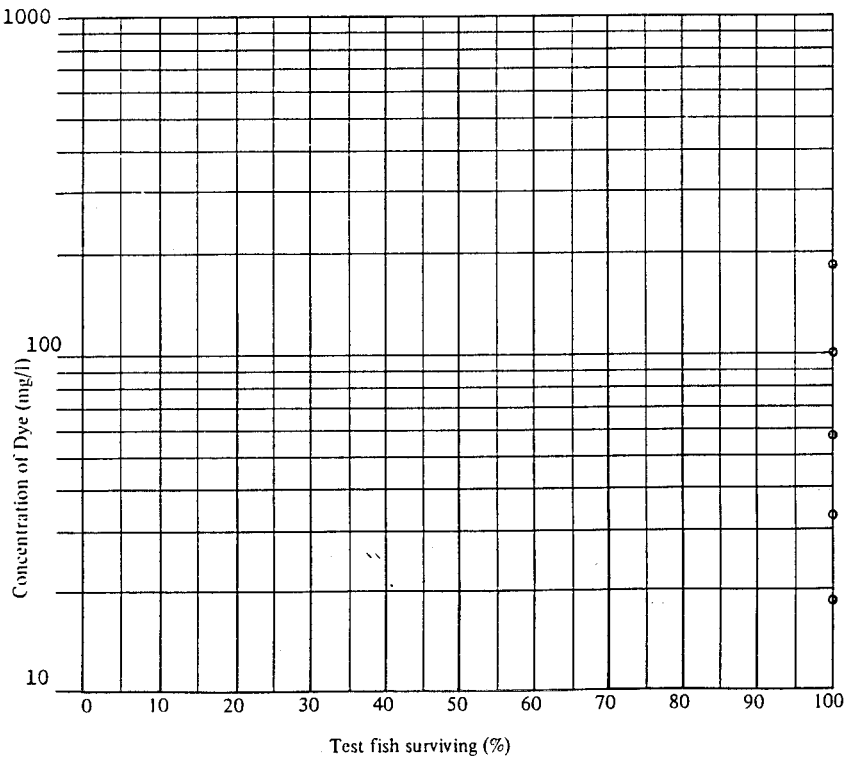
   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 6.5 mg/l; total dissolved solids 85 mg/l;

   turbidity 0 JTU; total alkalinity 19 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.0 to 18.2 C. Initial dissolved oxygen 5.8-8.1 mg/l; range during test, 4.3 to 8.1 mg/l. Initial pH 6.4-7.2 ; range during test, 6.4 to 7.2 .

7. 96-hour TL_{50}: >180 mg/l. (Aerated 5 min. each at 48 hr.)



Acclimation of fish: Fish received 2/14/72 maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C.

Pretreatment of fish: Treated with tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	11	11	11	11	11

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.4	8.1	6.6	6.9	7.0	6.4
18	7.0	7.2	6.6	6.0	7.1	6.2
32	7.0	7.1	6.6	5.5	7.0	6.0
56	7.0	7.0	6.6	5.4	7.0	5.8
100	7.1	7.1	6.7	5.0	7.0	5.5
180	7.2	7.2	6.8	5.2	7.1	5.6

Observations on behavior of fish during the tests: Not remarkable.

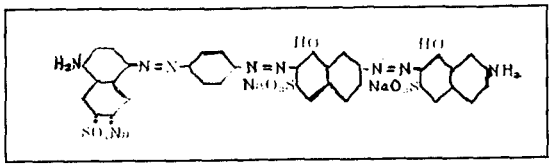
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Black 80 C. I. No. 31600 Date tested 3/2-3/6/72

   Supplier American Dye Manufacturers Inst. Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10-11

   Avg. wt. 0.56 g; range, 0.29 to 0.99 g. Avg. length 4.27 cm; range, 3.40 to 5.00 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 6.5 mg/l; total dissolved solids 85 mg/l;

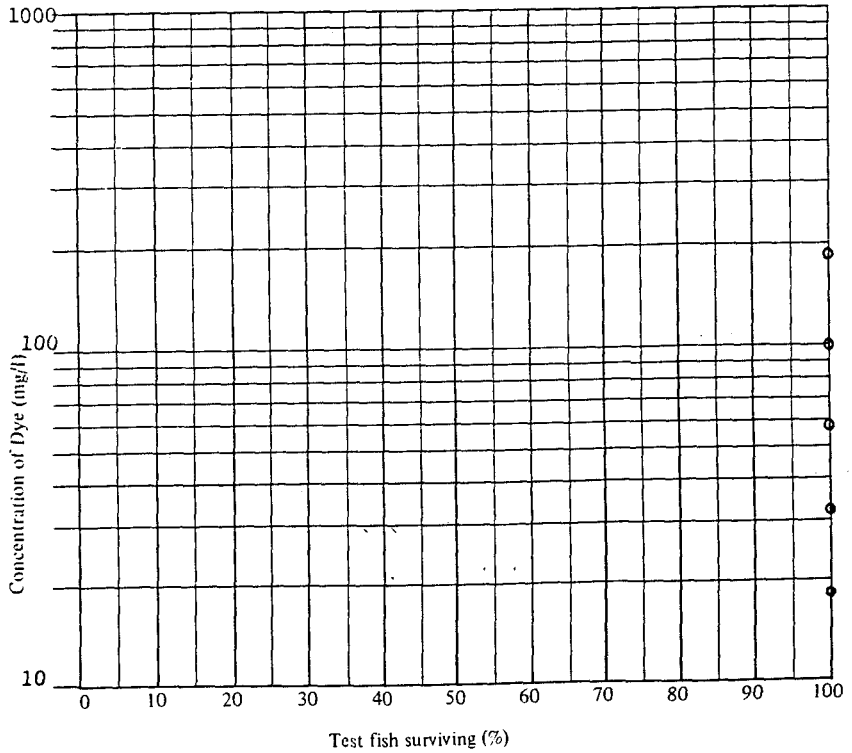
   turbidity 0 JTU; total alkalinity 19 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.0 to 18.2 C. Initial dissolved oxygen 7.0-8.1 mg/l; range during test, 5.0 to 7.2 mg/l. Initial pH 6.4-7.2 ; range during test, 6.4 to 7.2 .

   (Aerated 5 min. each at 48 hr.)

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish: Fish received October 20 maintained in plastic stock tank in con
cusly running charcoal-sand filtered Chapel Hill tap water, aerated with ai

Pretreatment of fish: Treated with tetracycline October 20-22; NaCl 11/1; tetracycline
Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	9
32	10	10	10	10	10
56	20	20	20	20	20
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.0	7.5	7.1	3.7	6.9	2.9
32	7.1	7.0	7.2	4.0	7.0	3.6
56	7.2	7.5	7.0	2.9	6.8	2.5
100	7.3	7.5	7.2	3.8	7.1	3.7
180	7.4	7.5	7.3	4.1	7.1	4.1

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Waste* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of procedures. Fish tests and their application are described in *Standard Methods* as follows:

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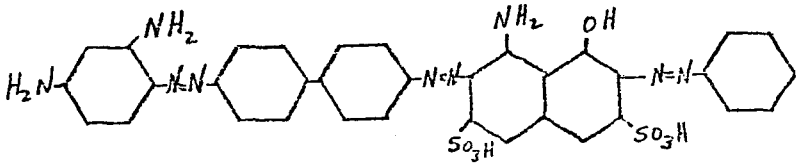
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Black 38 C. I. No. 30235 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10-20*

   Avg. wt. 1.0 g; range, 0.4 to 2.6 g. Avg. length 4.9 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

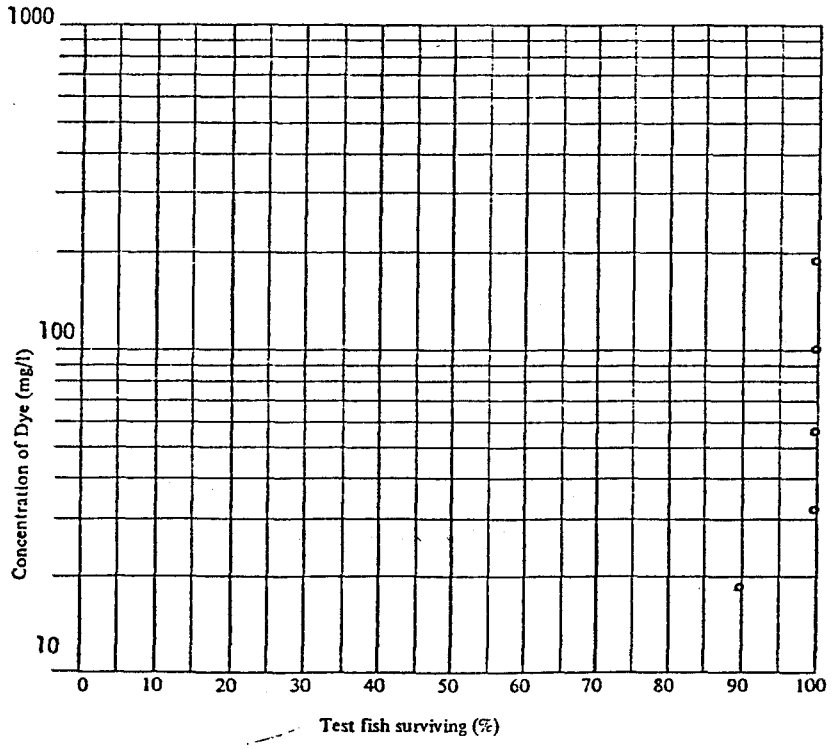
   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 17.1 to 17.5 C. Initial dissolved oxygen 7.0-7.5mg/l; range during test, 2.5 to 7.5 mg/l. Initial pH 7.1-7.4 ; range during test, 6.8 to 7.4 .

7. 96-hour TL_{50}: ≥180 mg/l.

*One jar was accidentally stocked twice.



Acclimation of fish: Fish received October 20 maintained in plastic stock tank in contir
cusly running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish: Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/
Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	9
32	10	10	10	10	10
56	20	20	20	20	20
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.0	7.5	7.1	3.7	6.9	2.9
32	7.1	7.0	7.2	4.0	7.0	3.6
56	7.2	7.5	7.0	2.9	6.8	2.5
100	7.3	7.5	7.2	3.8	7.1	3.7
180	7.4	7.5	7.3	4.1	7.1	4.1

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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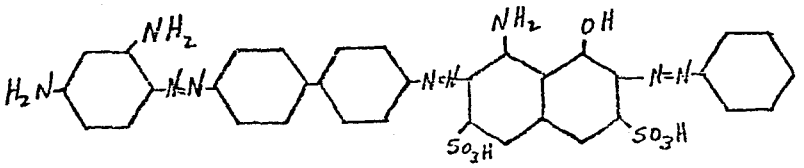
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Black 38 C. I. No. 30235 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10-20*

   Avg. wt. 1.0 g; range, 0.4 to 2.6 g. Avg. length 4.9 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1; TOC 34 mg/l; Fe 0.63 mg/l;

   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

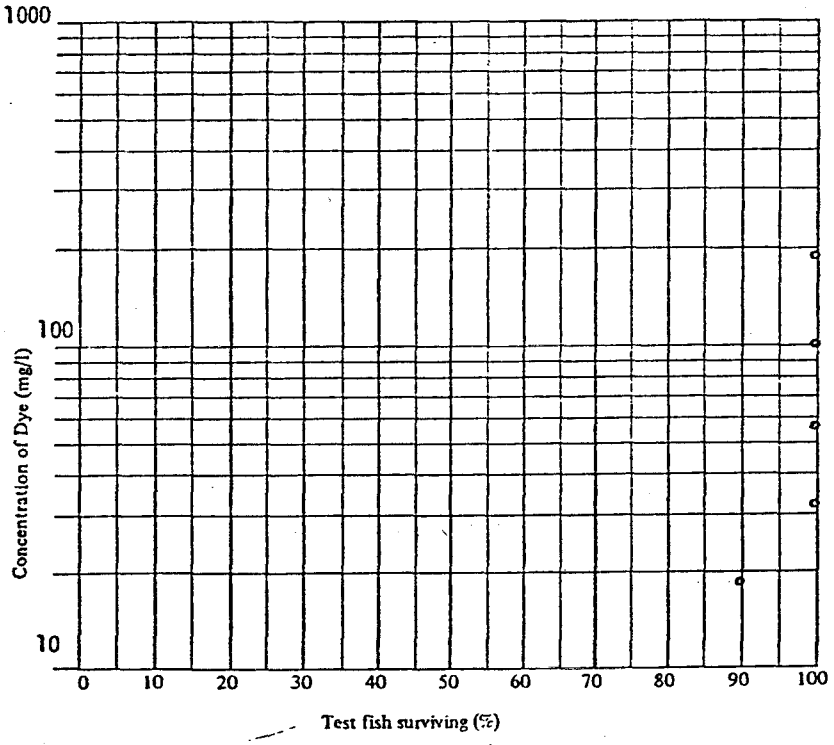
5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 17.1 to 17.5 C. Initial

   dissolved oxygen 7.0-7.5mg/l; range during test, 2.5 to 7.5 mg/l. Initial pH 7.1-7.4;

   range during test, 6.8 to 7.4.

7. 96-hour TL_{50}: >180 mg/l. *One jar was accidentally stocked twice.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.1	7.3	7.2	3.9	6.8	3.8
32	7.2	7.4	7.2	4.1	6.7	3.7
56	7.4	7.3	7.3	3.6	6.7	3.4
100	7.8	7.0	7.4	4.0	6.8	3.7
180	8.2	7.2	7.6	4.2	7.1	3.9

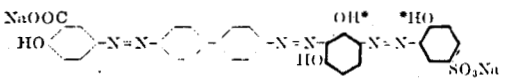
Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

Copper complex derived from



Dye formula

1. Test dye Direct Brown 95 C. I. No. 30145 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.2 g; range, 0.4 to 2.6 g. Avg. length 4.7 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material glass ; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

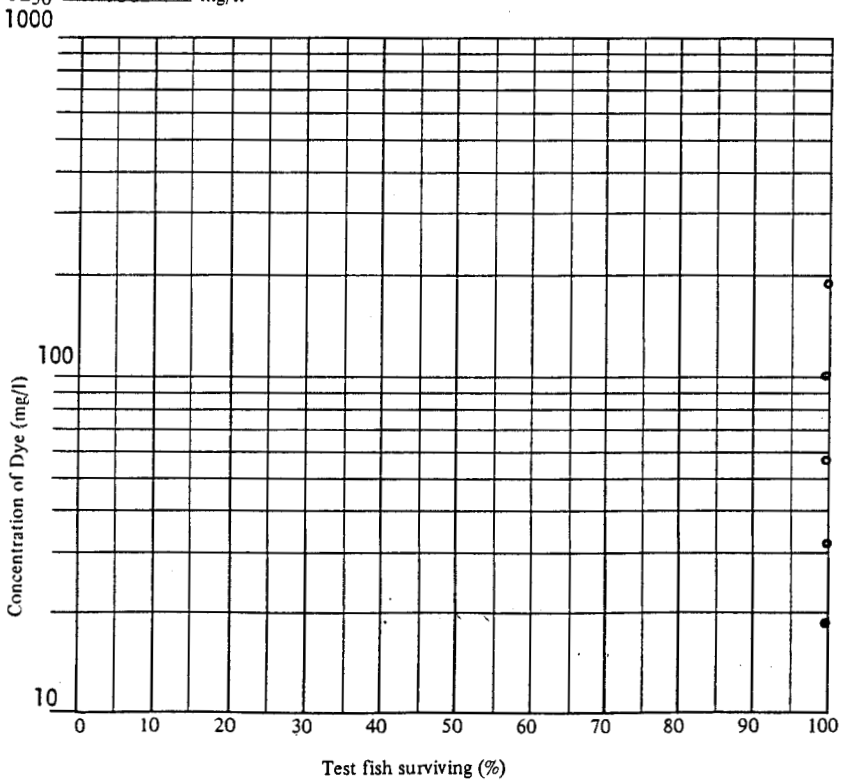
5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.9 to 17.5 C. Initial

   dissolved oxygen 7.0-7.5 mg/l; range during test, 2.8 to 7.5 mg/l. Initial pH 7.1-8.2 ;

   range during test, 6.7 to 8.2 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20, maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air.

Pretreatment of fish Treated with tetracycline Oct. 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	8
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.1	7.5	7.1	4.2	6.9	3.2
32	7.0	7.2	7.1	3.6	7.0	2.7
56	7.0	7.1	7.1	4.1	7.1	3.6
100	7.2	7.4	7.2	3.9	7.1	3.9
180	7.3	7.5	7.3	4.5	7.1	3.6

Observations on behavior of fish during the tests: Not remarkable

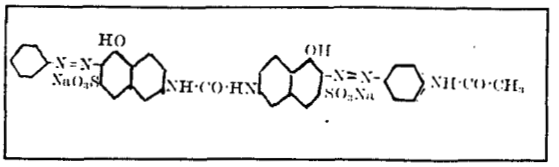
Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Red 23 C. I. No. 29160 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solution refrigerated.

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville, N. C. Number per jar 10

   Avg. wt. 1.4 g; range, 0.7 to 2.6 g. Avg. length 5.0 cm; range, 3.5 to 6.5 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

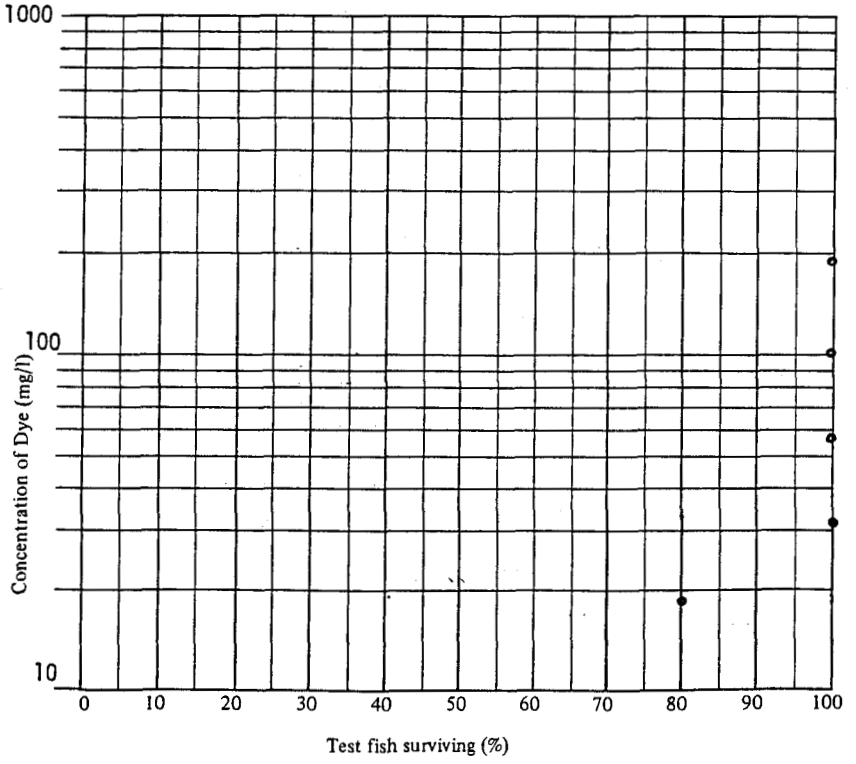
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.5 to 17.5 C. Initial dissolved oxygen 7.1-7.5 mg/l; range during test, 2.7 to 7.5 mg/l. Initial pH 7.0-7.3 ; range during test, 6.9 to 7.3 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	9	9	9
100	10	10	10	10	10
180	11	11	11	11	11

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.1	7.5	7.2	4.1	7.0	3.4
32	7.1	7.6	7.1	3.7	6.9	3.0
56	7.2	7.5	7.2	3.2	6.9	2.6
100	7.1	7.5	7.2	3.7	6.9	3.7
180	7.2	7.5	7.2	4.0	6.9	2.8

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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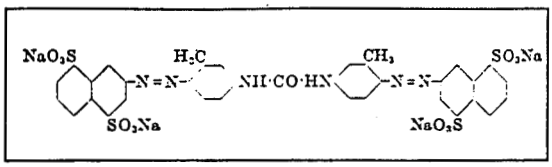
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

1. Test dye Direct Yellow 50 C. I. No. 29025 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10-11

   Avg. wt. 1.3 g; range, 0.4 to 2.6 g. Avg. length 6.1 cm; range, 4.0 to 7.0 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 : TOC 34 mg/l; Fe 0.63 mg/l;

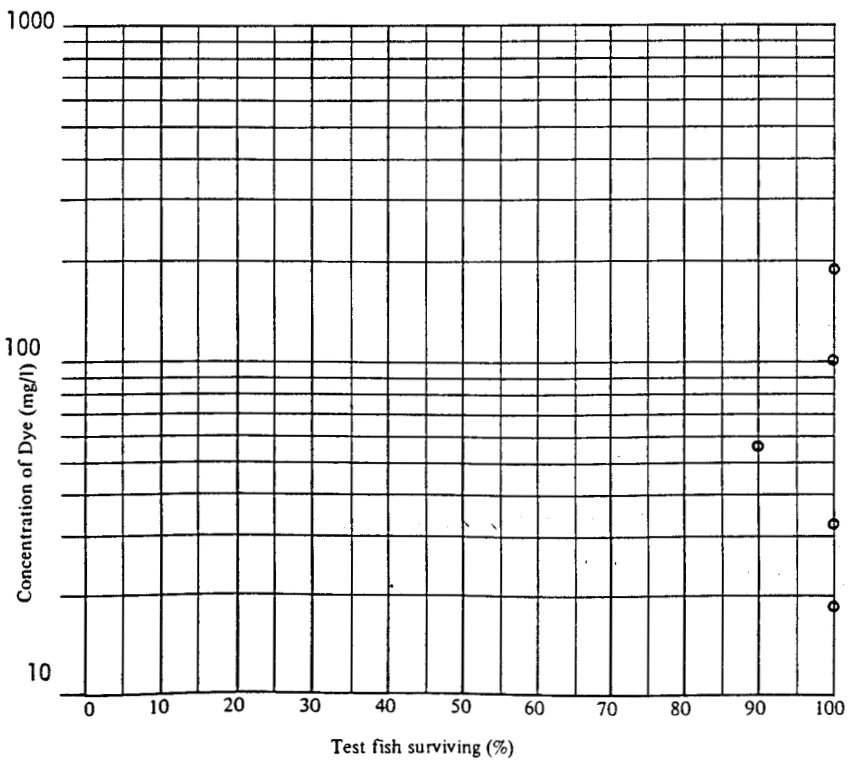
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.7 to 17.5 C. Initial dissolved oxygen 7.5-7.6 mg/l; range during test, 2.6 to 7.6 mg/l. Initial pH 7.1-7.2 ; range during test, 6.9 to 7.2 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air.

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.1	7.5	7.2	3.6	6.8	3.2
32	7.1	7.4	7.3	3.7	6.8	3.7
56	7.2	7.4	7.2	3.2	6.8	3.0
100	7.2	7.4	7.2	3.2	6.8	3.3
180	7.2	7.4	7.2	3.1	6.8	3.2

Observations on behavior of fish during the tests: Not remarkable

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UNC WASTEWATER | FISH BIOASSAY TESTS

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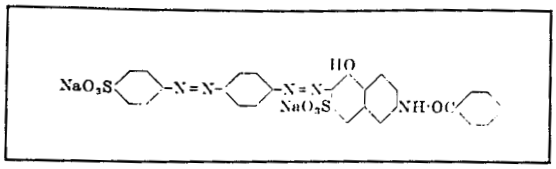


Dye formula

1. Test dye Direct Red 81 C. I. No. 28160 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solutions refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.3 g; range, 0.4 to 2.6 g. Avg. length 49 cm; range, 3.0 to 6.5 cm.

3. Test jars: Material glass ; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

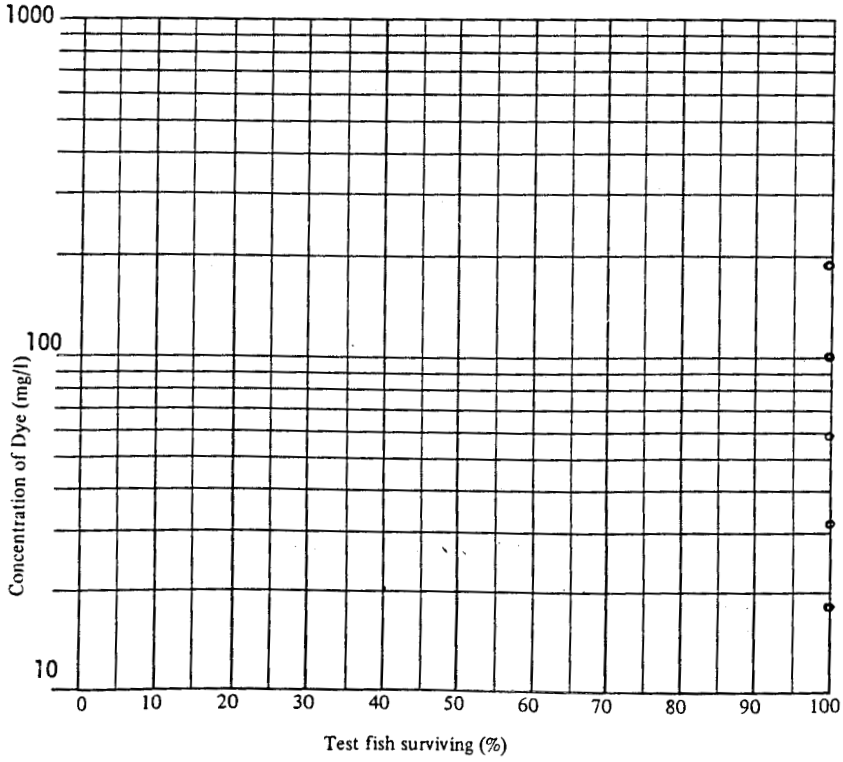
5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 15.0 to 17.5 C. Initial

   dissolved oxygen 7.4-7.5mg/l; range during test, 2.8 to 7.5 mg/l. Initial pH 7.1-7.2 ;

   range during test, 6.8 to 7.2 .

7. 96-hour TL_{50}: > 180 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
1	10	10	10	10	10
1.8	10	10	10	10	10
3.2	10	10	10	10	10
5.6	10	2	2	2	1
10	10	0	-	-	-

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.3	7.5	6.2	2.6	6.5	2.7
1	7.5	7.5	6.3	2.2	6.7	2.9
1.8	7.5	7.3	6.3	2.2	6.8	2.3
3.2	7.5	7.4	6.3	2.0	6.7	2.0
5.6	7.6	7.4	6.55	3.5	6.7	2.6
10	7.5	7.4	7.2	6.9	-	-

Observations on behavior of fish during the tests: fish dyed pale blue-violet at all concentrations

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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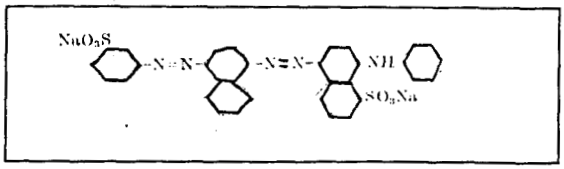


Dye formula

1. Test dye Acid Blue 113 C. I. No. 26360 Date tested 1/31/72

   Supplier ADMI Form supplied Dry powder

   Conditions of storage Stored at c. 20 C in dark

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 2.5 g; range, 0.85 to 5.13 g. Avg. length 6.5 cm; range, 4.90 to 8.20 cm.

3. Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.9 ; TOC 2 mg/l; Fe <0.2 mg/l;

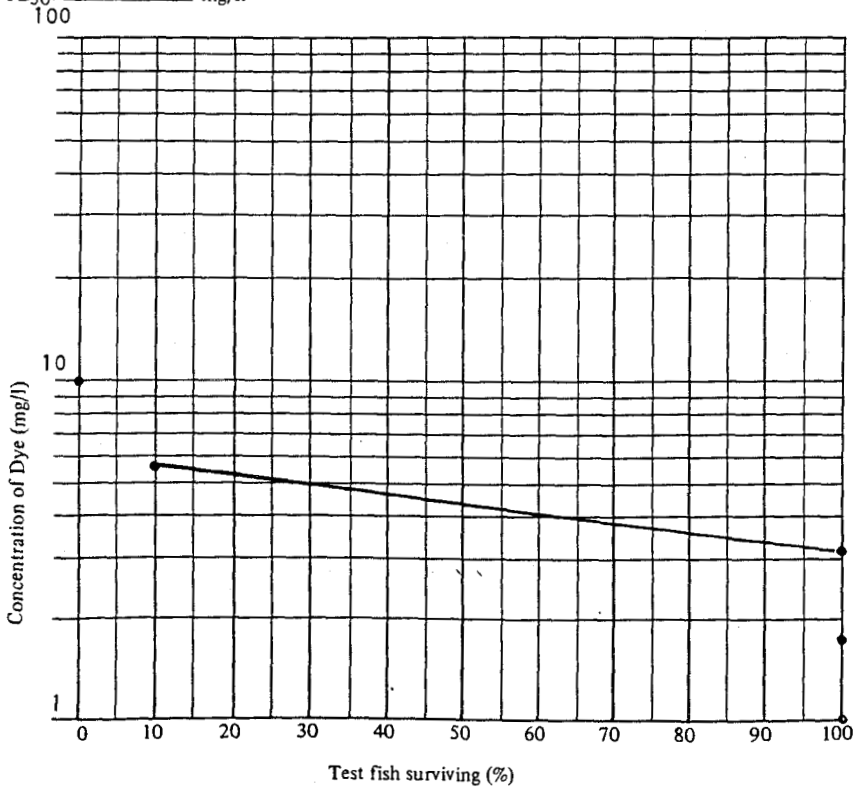
   Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l;

   turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

5. Procedure: Static

6. *Test conditions:* Temperature 15 C; range during test, 13.2 to 17.0 C. Initial dissolved oxygen 7.3-7.5 mg/l; range during test, 2.0 to 7.5 mg/l. Initial pH 7.3-7.6 ; range during test, 6.2 to 7.6 (Aerated for 5 min each at 48 hr)

7. 96-hour TL_{50}: 4 mg/l.



Acclimation of fish Fish received 12/13/71. Maintained in plastic stock tank receiving constant flow of charcoal and sand filtered Chapel Hill tap water.

Pretreatment of fish Treated with tetracycline HCL on 12/13/71 and 1/24/72.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
10	10	10	10	10	10
18	10	10	10	10	9
32	10	0	-	-	-
56	10	0	-	-	-
100	10	0	-	-	-

TEST CONDITIONS

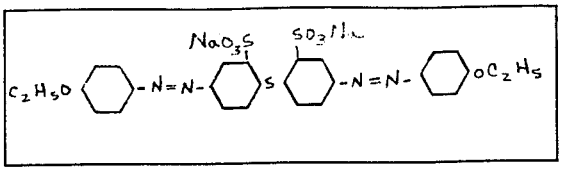
Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.3	7.5	6.2	2.6	6.5	2.7
10	7.5	7.6	6.4	3.2	6.8	3.0
18	7.6	7.4	6.4	3.5	6.7	2.5
32	7.6	7.4	7.2	7.0	-	-
56	7.6	7.4	7.4	7.0	-	-
100	7.6	7.4	7.5	7.0	-	-

Observations on behavior of fish during the tests: Surviving fish not dyed. Dead fish dyed golden yellow.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

Test dye Acid Yellow 38 C. I. No. 25135 Date tested 1/31/72

Supplier ADMI Form supplied Dry powder

Conditions of storage Stored at c. 20 C in dark

Test fish Fathead Minnow (Pimephales promelas)

Supplier Windmill Fish Hatcheries Number per jar 10

Avg. wt. 2.6 g; range, 1.00 to 5.48 g. Avg. length 6.5 cm; range, 5.00 to 7.90 cm.

Test jars: Material Glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

Dilution water Charcoal-sand filtered Chapel Hill tap water

Volume per jar 15 l; depth in jar 30 cm; pH 6.9 ; TOC 2 mg/l; Fe <0.2 mg/l;

Al <0.5 mg/l; Mg 1.2 mg/l; Ca 8.7 mg/l; total dissolved solids 129 mg/l;

turbidity 0 JTU; total alkalinity 30 mg/l as $CaCO_3$.

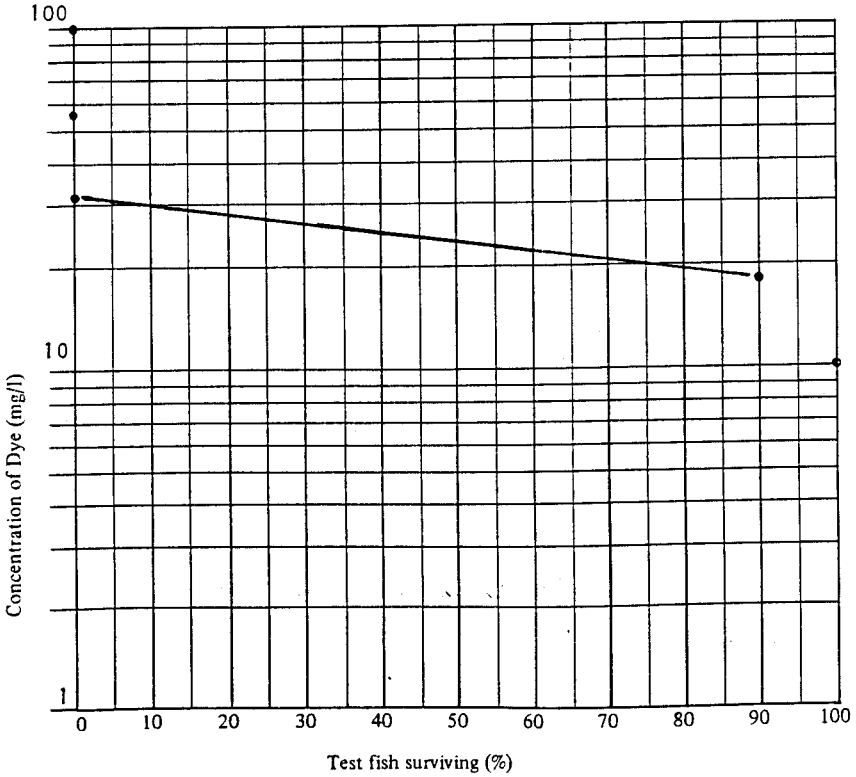
Procedure: Static

Test conditions: Temperature 15 C; range during test, 13.9 to 17.0 C. Initial

dissolved oxygen 7.4-7.6 mg/l; range during test, 2.5 to 7.6 mg/l. Initial pH 7.3-7.6 ;

range during test, 6.2 to 7.6 . (aerated for 5 min each at 48 hr.)

96-hour TL_{50}: 23 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flow, aerated, charcoal-sand filtered Chapel Hill tap water 14-18 C

Pretreatment of fish Treatment with tetracycline HCl (Tetrachel) 2/14/72 and 3/17/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	-	-	-	9
100	10	8	8	8	8
180	10	6	1	0	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.2	6.8			6.6	5.2
18	7.2	7.6			6.4	5.1
32	7.2	7.5			6.5	5.2
56	7.3	7.2			6.4	5.5
100	7.5	7.1			6.6	4.9
180	8.0	7.2			6.6	-

Observations on behavior of fish during the tests: All fish dyed very pale yellow.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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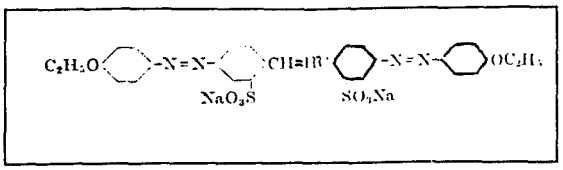
Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate information on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an accurate estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in water with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures of the relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent concentrations that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, and still lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and growth, resistance to disease, reproductive capacity or ability to compete with other species of the biota."



Dye formula

Test dye Direct Yellow 12 C. I. No. 24895 Date tested 3/23-3/27/72

Supplier American Dye Manufactures Inst Form supplied Dry powder

Conditions of storage Stored in dark at approximately 20 C

Test fish Fathead minnow (Pimephales promelas)

Supplier Windmill Fish Hatcheries Number per jar 10

Avg. wt. 0.60 g; range, 0.36 to 1.25 g. Avg. length 4.30 cm; range, 3.60 to 5.70 cm.

Test jars: Material glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

Dilution water charcoal- and filtered Chapel Hill tap water

Volume per jar 15 l; depth in jar 30 cm; pH 7.3; TOC 5 mg/l; Fe <0.02 mg/l;

Al <1.0 mg/l; Mg 1.6 mg/l; Ca 5.1 mg/l; total dissolved solids 127 mg/l;

turbidity 2 JTU; total alkalinity 22 mg/l as $CaCO_3$.

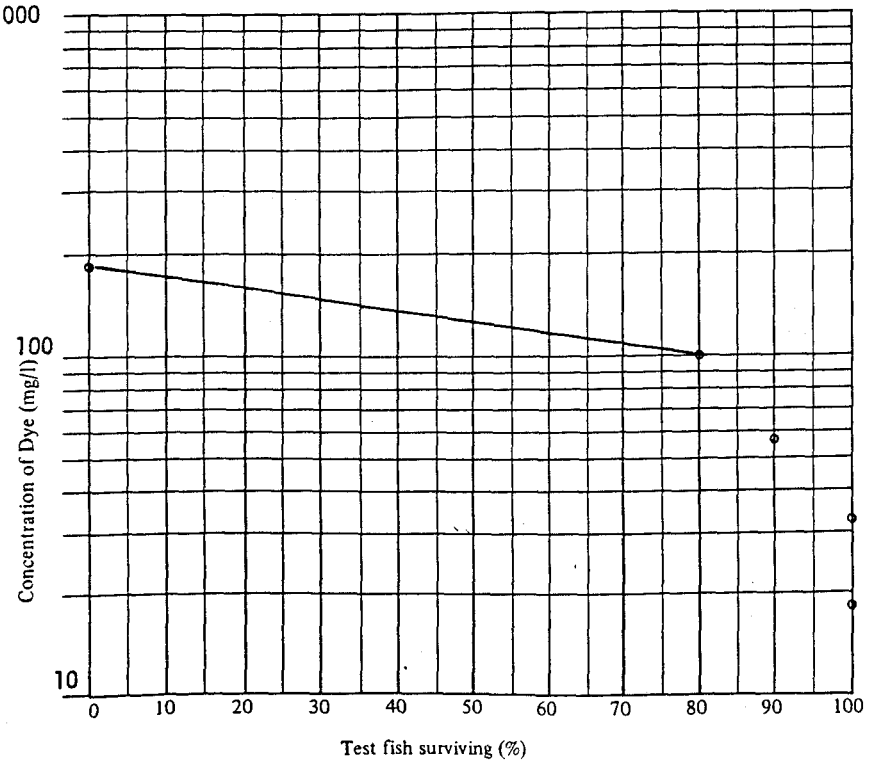
Procedure: Static

Test conditions: Temperature 15 C; range during test, 14.1 to 16.0 C. Initial

dissolved oxygen 6.8-7.6 mg/l; range during test, 4.9 to 7.6 mg/l. Initial pH 7.2-8.0;

range during test, 6.4 to 8.0.

96-hour TL_{50}: 125 mg/l.



Acclimation of fish Fish received 2/14/72; maintained in constantly flowing, aerated, charcoal-sand filtered Chapel Hill tap water at 14-18 C

Pretreatment of fish Treated with tetracycline HCl (Tetrachel) 2/14/72

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	10
18	10	10	10	9	9
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	6.92	7.5	6.81	6.2	6.70	6.6
18	7.09	7.4	6.85	6.5	6.72	6.7
32	7.08	7.4	6.92	6.3	6.73	6.3
56	7.04	7.5	6.94	6.2	6.68	6.2
100	7.02	7.2	6.83	5.9	6.72	6.1
180	7.00	7.6	6.81	5.9	6.68	5.4

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, determine permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and chemical variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by water pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions."

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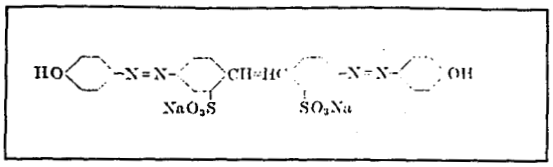
UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Yellow 4 C. I. No. 24890 Date tested 3/6-3/10/72

   Supplier American Dye Manufacturers Inst Form supplied Dry powder

   Conditions of storage Stored in dark at approximately 20 C

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Windmill Fish Hatcheries Number per jar 10

   Avg. wt. 0.58 g; range, 0.34 to 1.45 g. Avg. length 4.22 cm; range, 3.20 to 5.70 cm.

3. Test jars: Material glass ; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 6.8 ; TOC 4 mg/l; Fe <0.02 mg/l;

   Al <1.0 mg/l; Mg 1.4 mg/l; Ca 5.5 mg/l; total dissolved solids 59 mg/l;

   turbidity 0 JTU; total alkalinity 15 mg/l as $CaCO_3$.

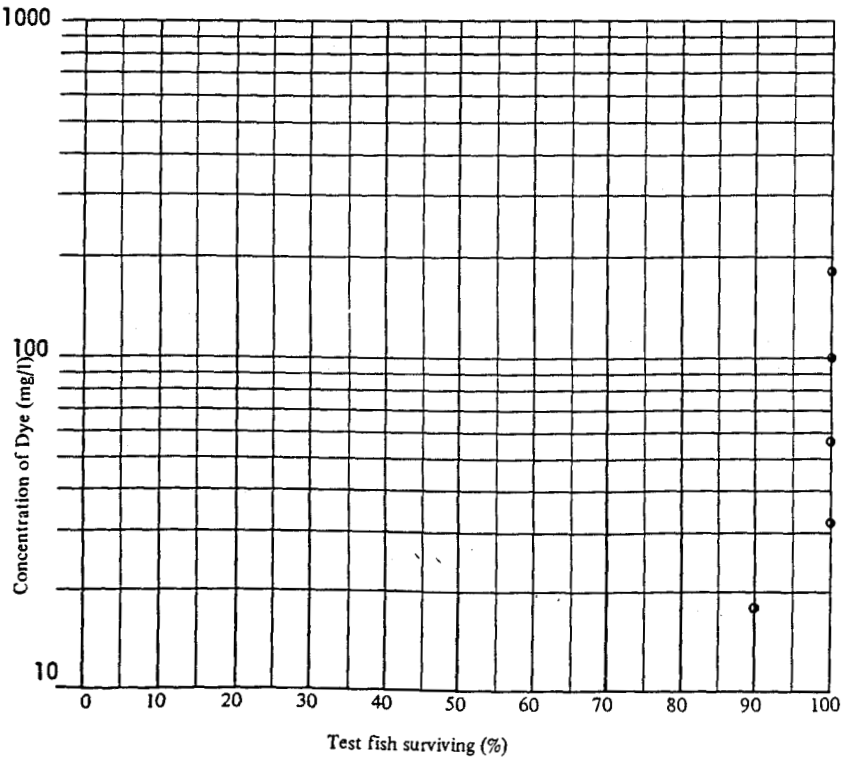
5. Procedure: Static

6. Test conditions: Temperature 15 C; range during test, 13.4 to 17.2 C. Initial

   dissolved oxygen 7.2-7.6 mg/l; range during test, 5.4 to 7.2 mg/l. Initial pH 6.9-7.1 ;

   range during test, 6.7 to 7.1 .

7. 96-hour TL_{50}: >180 mg/l. (Aerated 5 min each at 48 hr)



Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	10
56	10	10	10	10	10
100	10	10	10	10	10
180	10	10	10	10	6

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.2	7.4	7.2	3.4	6.9	3.5
32	7.4	7.4	7.3	3.6	6.8	3.5
56	7.9	7.5	7.3	3.5	6.9	3.0
100	8.3	7.5	7.5	3.4	7.0	3.0
180	8.5	7.5	8.1	3.7	7.2	3.0

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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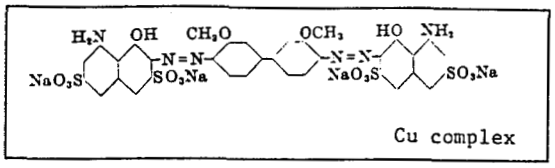
UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Blue 218 C. I. No. 24401 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solutions refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.3 g; range, 0.5 to 2.6 g. Avg. length 5.2 cm; range, 4.0 to 6.5 cm.

3. Test jars: Material glass ; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

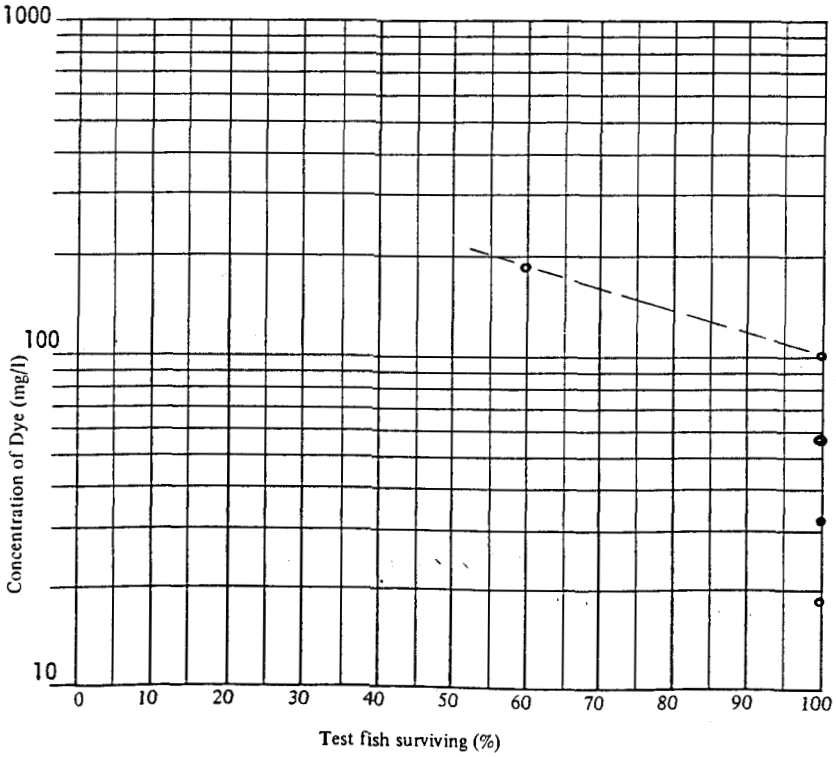
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.5 to 17.5 C. Initial dissolved oxygen 7.4-7.5 mg/l; range during test, 2.8 to 7.5 mg/l. Initial pH 7.1-8.5 ; range during test, 6.8 to 8.5 .

7. 96-hour TL_{50}: >180 mg/l.



The University of North Carolina / Department of Environmental Sciences and Engineering
Chapel Hill, North Carolina

Acclimation of fish Fish received October 20 maintained in plastic stock tank in continuously running charcoal-sand filtered Chapel Hill tap water, aerated with air

Pretreatment of fish Treated with tetracycline October 20-22; NaCl 11/1; tetracycline 11/2. Starved for 48 hr prior to experiment

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr.
0	10	10	10	10	9
18	10	10	10	10	10
32	10	10	10	10	9
56	10	10	10	10	10
100	10	10	10	10	9
180	10	10	10	10	10

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.5	7.0	3.6	7.0	2.8
18	7.2	7.5	7.1	3.6	7.0	3.6
32	7.3	7.3	7.3	4.2	7.1	4.1
56	7.3	7.0	7.2	3.9	7.0	3.7
100	7.4	7.3	7.2	3.6	6.9	1.9
180	7.4	7.4	7.3	3.7	7.1	3.6

Observations on behavior of fish during the tests: Not remarkable

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Wastewater*, 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of these procedures. Fish tests and their application are described in *Standard Methods* as follows:

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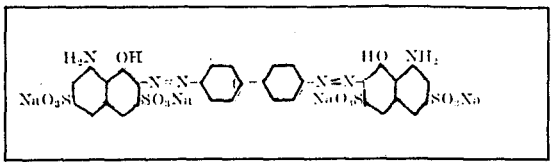
UNC WASTEWATER | FISH BIOASSAY TESTS

RESEARCH CENTER | AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Direct Blue 6 C. I. No. 22610 Date tested 11/11-15/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage composite stored at 20 C in dark. Stock solution refrigerated

2. Test fish Fathead Minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.2 g; range, 0.4 to 2.6 g. Avg. length 5.1 cm; range, 3.9 to 6.5 cm.

3. Test jars: Material glass ; capacity 20 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Chapel Hill tap water, activated charcoal-sand filtered

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1 ; TOC 34 mg/l; Fe 0.63 mg/l;

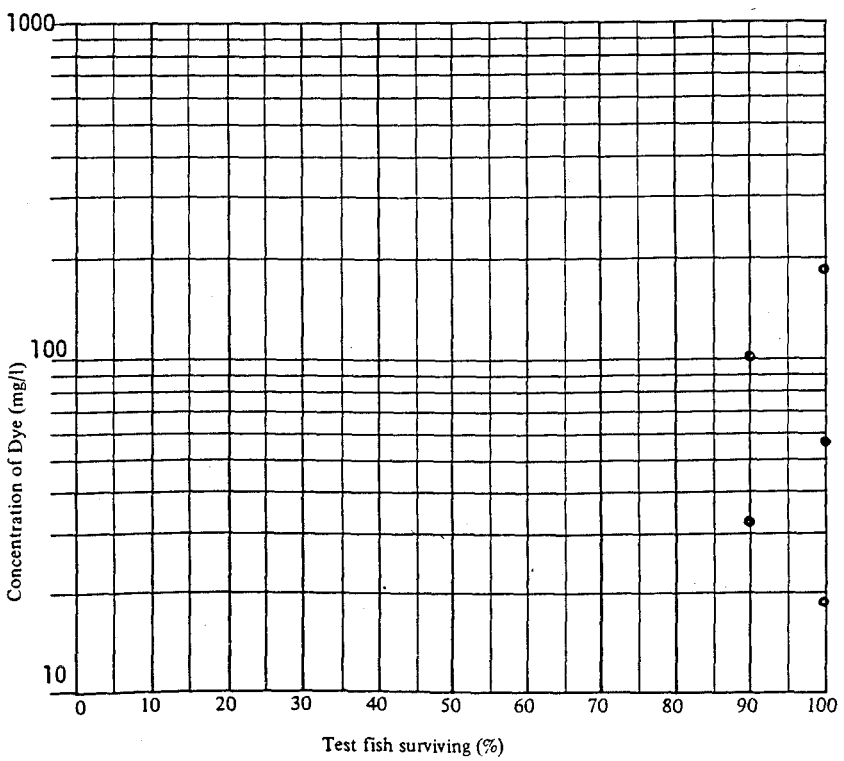
   Al <0.1 mg/l; Mg 1.0 mg/l; Ca 8.6 mg/l; total dissolved solids 138 mg/l;

   turbidity 0 JTU; total alkalinity 32 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 17 C; range during test, 16.5 to 17.5 C. Initial dissolved oxygen 7.0-7.5 mg/l; range during test, 1.9 to 7.5 mg/l. Initial pH 7.1-7.4 ; range during test, 6.9 to 7.4 .

7. 96-hour TL_{50}: >180 mg/l.



Acclimation of fish Fish received October 20, 1971. Maintained in 250 gal. plasti
receiving constant flow of filtered tap water and aerated with compressed ai
Temp. 15-20 C.

Pretreatment of fish Treated on October 20-22, November 1, and Nov. 16-18, 1971 with
chel (Tetracycline H Cl) to control disease. Treated with Na Cl 11/1. Star
48 hr prior to experiment.

EXPERIMENTAL DATA

Dye Concentration mg/l	No. test fish	No. of fish surviving			
		24 hr.	48 hr.	72 hr.	96 hr
0	10	10	9	9	9
1.0	10	10	10	9	9
1.8	10	10	10	8	8
3.2	10	10	10	8	7
5.6	10	10	9	7	5
10.0	10	10	1	1	0

TEST CONDITIONS

Dye Concentration mg/l	0 hr.		48 hr.		96 hr.	
	pH	DO (mg/l)	pH	DO (mg/l)	pH	DO (mg/l)
0	7.1	7.0	6.6	4.4	6.4	3.6
1.0	7.1	7.0	6.5	4.5	6.5	4.3
1.8	7.1	7.0	6.5	4.6	6.6	4.4
3.2	7.1	6.8	6.4	4.5	6.6	4.5
5.6	7.1	6.8	6.4	4.1	6.5	4.6
10.0	7.0	7.2	6.5	5.1	6.6	4.5

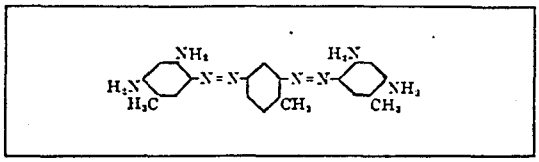
Observations on behavior of fish during the tests: All fish dyed deep orange from first day. Accumulation of brown droppings noted. At concentrations of 3.2 mg/l and hig fish tended to stay at the top of the jars. Surface scum with appearance of noted in all jars.

Fish bioassay tests were conducted according to procedures described in *Standard Methods for the Examination of Water and Waste* 13th ed., APHA, AWWA, WPCF, New York, N. Y. (1971). The tests are subject therefore to the applications and limitations of procedures. Fish tests and their application are described in *Standard Methods* as follows:

Principles and application of fish bioassay tests: "Bioassays are conducted to evaluate the toxicity of effluents or other materials, det permissible effluent discharge rates, establish the relative sensitivity of various fish species, and identify effects of physical and ch variables such as temperature and pH on toxicity. Bioassays can be used to judge compliance with water quality standards established by pollution control authorities. In a bioassay, experimental organisms are subjected to a series of concentrations of a known or suspected to under adequately controlled conditions."

Accuracy, interpretation and limitations: "Toxicity studies with a randomly selected species cannot be expected to give accurate infor on the toxicity of that material to other species and life stages or to an entire biota. A toxicity bioassay with one species yields an ac estimate of the toxicity of the material in question only to others of that species of similar size, age and physiological condition and in with the same or similar characteristics and under similar test conditions. . . . The 48-hr and 96-hr TL_{50} values provide useful measures relative acute lethal toxicity of tested substances to fish under certain experimental conditions, but these values do not represent con tions that are safe in fish habitats. Long-term exposure to much lower concentrations may be lethal to fishes and other organisms, ar lower concentrations may cause nonlethal impairment of their functions or performance, such as swimming ability, appetite and g resistance to disease, reproductive capacity or ability to compete with other species of the biota."

UNC WASTEWATER	FISH BIOASSAY TESTS
RESEARCH CENTER	AMERICAN DYE MANUFACTURERS INSTITUTE PROJECT



Dye formula

1. Test dye Basic Brown 4 C. I. No. 21010 Date tested 11/29-12/3/71

   Supplier ADMI Form supplied composite, dry powder

   Conditions of storage Stored dry at c. 20 C in dark; stock solutions refrigerated in glass

2. Test fish Fathead minnow (Pimephales promelas)

   Supplier Berry Water Gardens, Kernersville Number per jar 10

   Avg. wt. 1.4 g; range, 0.4 to 2.8 g. Avg. length 5.5 cm; range, 3.5 to 7.5 cm.

3. Test jars: Material Glass; capacity 19 l; dimensions 25 cm(d) x 47 cm(h).

4. Dilution water Charcoal-sand filtered Chapel Hill tap water

   Volume per jar 15 l; depth in jar 30 cm; pH 7.1; TOC 3 mg/l; Fe <0.01 mg/l;

   Al <0.1 mg/l; Mg 0.53 mg/l; Ca 8.6 mg/l; total dissolved solids 121 mg/l;

   turbidity 0 JTU; total alkalinity 29 mg/l as $CaCO_3$.

5. Procedure: Static

6. Test conditions: Temperature 20 C; range during test, 17.2 to 20 C. Initial dissolved oxygen 6.8-7.2 mg/l; range during test, 3.6 to 7.2 mg/l. Initial pH 7.0-7.1; range during test, 6.4 to 7.1. (Aerated for 5 min. each at 48 hr.)

7. 96-hour TL_{50}: 5.6 mg/l.