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67. Effects of Water Hardness on the Toxicity of Several Organic and Inorganic Herbicides to Fish

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CONTENTS

	Page
Abstract	3
Methods	5
Results	6
Silvex	6
2, 4 D	9
Endothall	10
Pentachlorophenol	13
Dichlobenil	14
Sodium arsenite	15
Copper sulfate	15
Discussion	16
Conclusion	20
Acknowledgments	20

EFFECTS OF WATER HARDNESS ON THE TOXICITY OF SEVERAL ORGANIC AND INORGANIC HERBICIDES TO FISH

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ABSTRACT.--Effects of water hardness on the acute toxicity of organic and inorganic herbicides were determined in static bioassays. Concentrations of total hardness (calculated as $CaCO_3$) of 13.0, 52.2, 208.7, and 365.2 ppm were tested in water containing calcium/magnesium ion ratios of 1:1 and 5:1. Bluegills were the principal test species; rainbow trout, bluespotted sunfish, goldfish, redear sunfish, and black bullheads were also tested. Organic herbicides tested included three formulations of 2, 4-D (BEE, PGBEE, DMS), three formulations of endothall (Na salt, and two dimethylalkylamine derivatives), and one formulation each of silvex (BEE), pentachlorophenol, and dichlobenil; inorganic herbicides included technical grades of sodium arsenite and copper sulfate. Hardness had no significant effect on toxicity of the organic herbicides or that of sodium arsenite; the toxicity of copper sulfate decreased in the harder waters. The significance of the results is discussed.

The utility of herbicides as tools in the management of fisheries is dependent largely on their toxicities to fish and fish-food organisms. The acute toxicity of many herbicides proposed for use in aquatic weed control has been determined for a number of fish and invertebrates, but little information is available on the effects of several environmental factors which may increase or decrease the suitability of these herbicides as management tools.

Environmental factors, acting singly or in combination, that may affect the biological activity of aquatic herbicides include temperature, pH, dissolved gases, dissolved salts, turbidity, alkalinity, organic compounds, and hardness. We concentrated our research on one of these factors, water hardness, and its influence on the toxicity of seven herbicides to six species of fish.

Several authors have presented data on the effect of water hardness on the toxicity of organic herbicides. Webb (1959) found that aminotriazole, Phygon X-L (2, 3-dichloro-1, 4-napthoquinone), and Sinox General (a dinitrophenol compound) were slightly more toxic to redsided shiners (Richardsonius balteatus) in soft water. He concluded that pH, alkalinity, and hardness have no major effect on the toxicity of the compounds tested. Davis and Hardcastle (1959), working with only slight differences in hardness, reported six herbicides to be less toxic to bluegills and largemouth bass in soft water. Three of

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the six herbicides included the dimethylamine salt of the phenoxy compounds 2, 4-D, 4-MCPB, and 4-(2, 4-DB). Truchelut and Williams (1960a) reported that concentrations of up to 525 parts per million of calcium ion (1311 ppm hardness) enhanced the activity of unidentified esters of silvex and 2, 4-D against coontail (*Ceratophyllum demersum*) in greenhouse experiments. Surber and Pickering (1962) reported a generally greater toxicity to a minnow and two species of centrarchids in soft water for six herbicides including disodium endothall, dalapon, diquat, two hyamine compounds, and two formulations of silvex. In a study of the toxicology of two formulations of endothall (disodium salt and a "coco" amine salt), Walker (1963) reported that the 96-hour TLm for four species of warm water fish was reduced slightly in hard water. Funk and Gaufin (1965) reported reduced effectiveness of several organic herbicides, including three amine formulations of endothall, as well as that of copper sulfate in "hard" waters with alkalinity over 300 ppm.

The two most widely used inorganic herbicides are sodium arsenite and copper sulfate, the latter being used primarily as an algicide. Published data on the interaction between hardness and arsenic compounds, particularly sodium arsenite, are not available. In an extensive review of the literature on the toxicity of heavy metals, Doudoroff and Katz (1953) cited 43 references on toxicity of copper to fish, 11 of which dealt with the interaction between hardness and copper sulfate. These authors point out the conflicting nature of much of the data on this interaction and concluded that the data showed only that copper, in concentrations between 0.025 ppm and 1.0 ppm as the ion, could be rapidly fatal to fishes in most natural fresh waters of the United States. Surber (1961) cited unpublished data of J. R. Anderson recording 48-hour TLm values for copper sulfate to bluegills of 0.6, 8.0, 10.0, and 45.0 ppm at total hardness of 15, 68, 100, and 132 ppm, respectively.

The literature thus reveals conflicting results concerning the effect of water hardness on the toxicity of herbicides. In most of the bioassay investigations cited above, natural surface waters were used as diluents, and either the chemical characteristics of the diluents were inadequately delineated or the reported variation in hardness was also associated with variations in pH and/or alkalinity. The reported effects may have been related to changes in one or any combination of these three parameters.

The concept of water hardness is deeply embedded in investigations involving water quality or aquatic ecology. In spite of its wide use by both the scientific and the nonscientific community, this property of water has been poorly defined and may account for the conflicting results apparent in the literature cited. Hardness is influenced by variations in pH, alkalinity, dissolved carbon dioxide, and dissolved solids. The oldest method of measuring hardness developed from its characteristic reduction of sudsing of soap. However, soap reacts with other substances such as heavy metals, acids, and certain organic compounds to give a false hardness value. The most widely used measurement of hardness is based on the pH-alkalinity relation of the dissolved carbon dioxide-bicarbonate-carbonate system and is usually reported as phenolphthalein and/or methyl orange hardness. More recently, hardness has been defined in terms of the presence of cations of the alkaline earths, primarily calcium or magnesium ions.

For the purpose of this investigation, hardness is defined as the characteristic of water represented by the total concentration of ions of the alkaline earths, expressed as parts per million of calcium carbonate. Calcium and magnesium ions were the only materials contributing to hardness of the diluents used in this study.

METHODS

By static bioassays, differences in the acute toxicities of several aquatic herbicides were determined at three or four levels of hardness. Acute toxicity was measured by estimation of the median lethal concentration (LC50) at standard time intervals. The LC50 is defined as the concentration of toxicant which produces a 50 percent mortality at standard time intervals and is expressed in parts per million (mg/l) of active ingredient of the toxicant. Because the LC50 values were determined without renewal of the toxicant, these values were based on initial concentrations only and do not account for losses due to volatilization, adsorption, precipitation, etc. All tests were run for 96 hours, and accumulative mortality data were recorded, when possible, at 6, 12, 24, 48, 72, and 96 hours.

The tests were conducted in 5-gallon wide-mouth jars, each containing 15 liters of test solution. The jars were partially immersed in water baths capable of maintaining standard temperatures within ± 1.0° C (fig. 1). Ten or twenty fish were exposed at each of five concentrations of the herbicide with a maximum of 10 fish per jar. Concentrations of toxicant were made up in logarithmic series as recommended by the American Public Health Association (1960). Tests were run in concurrent series of three, and sometimes four, levels of hardness. Controls were run for each level of hardness in all tests and were treated in exactly the same manner as the test fish with the exception of the lack of toxicant. Loss of control fish was practically nil, amounting to less than 0.1 percent of the total number of controls used throughout the study. Potential differences in resistance of test fish due to variations between batches, or to extended periods of holding in the laboratory, were controlled by use of a reference bioassay using *p, p'*-DDT. In general, these reference values were within the normal ranges reported in the literature. The reference check was carried out in water with a total hardness of 52.2 ppm.

Species used in the bioassays included rainbow trout (*Salmo gairdneri*), goldfish (*Carassius auratus*), black bullhead (*Ictalurus melas*), bluespotted sunfish (*Enneacanthus gloriosus*), bluegills (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*). The bluespotted sunfish and black bullheads were seined from ponds on the Patuxent Wildlife Research Center, Laurel, Md. Bluegills, redear sunfish, and goldfish were obtained from either the Harrison Lake National Fish Hatchery, Charles City, Va., or the Edenton National Fish Hatchery, Edenton, N. C. Rainbow trout were obtained from the Bowden National Fish Hatchery, Elkins, W. Va. Sizes of fish used and loading levels are presented in discussions of each herbicide.

All test fish were treated within 1 to 3 days after delivery to the laboratory with 1 ppm of neutral acriflavin and 20 ppm of formalin for 24 hours to control parasites and other diseases. After treatment, fish were held in aerated tap water in 8-foot plastic pools in the laboratory for up to 4 weeks, or until they were used for testing. Commercial fish food was fed at regular daily or twice-daily intervals. Three days prior to testing, several groups of 250-300 fish were acclimated in plastic aquariums to the temperature and diluent water to be used in the bioassay. Fish were randomly selected for acclimation and subsequent testing. The standard temperature for all tests with warm-water species was 24° C; rainbow trout were tested at 13° C. The fish were not fed during the 3-day acclimation period and the subsequent 4-day test period. Fish were placed in test jars approximately 1 to 3 hours before adding the herbicide to the diluent water. Any fish showing signs of distress (usually due to handling) were replaced prior to the start of the test. Conductivity and pH were

Figure 1:--Bioassay jars in place in temperature control bath with acclimating aquariums in background.

measured just before adding the toxicant. Test solutions were not aerated during the test period to reduce loss of volatile toxicants. Dissolved oxygen, measured at the beginning and end of the test, did not fall below 3 ppm in any bioassay. The number of dead fish was recorded at standard time intervals, and the observed mortalities were plotted on log-probability paper using the method of Litchfield and Wilcoxon (1949) to estimate the LC50.

Water used in all bioassays was reconstituted from deionized water having a resistivity of 100,000 ohms or greater. Two types of diluents designated Laurel No. 1 and Laurel No. 2, were used. Laurel No. 1 was prepared at four hardnesses with an approximate 1:1 ratio of calcium ion to magnesium ion (table 1). Laurel No. 2 water, with a Ca to Mg ion ratio of approximately 5:1, was prepared at three levels of hardness, corresponding to the three higher levels of Laurel No. 1 water (table 2).

The calcium-magnesium ion ratios of 1:1 and 5:1 were selected as representing the ranges of these ratios commonly found in most natural surface waters of North America (Hem, 1959). The hardness levels were likewise selected to represent ranges of hardness commonly found at many national fish hatcheries (Warren, 1963) and fishery research laboratories of the Bureau of Sport Fisheries and Wildlife, as well as being reported by Hem (1959).

Organic herbicides tested included various formulations of the following compounds: 2-(2,4,5-trichlorophenoxy) proprionic acid (silvex); 2,4-dichlorophenoxyacetic acid (2,4-D); 7-oxabicyclo-[2.2.1] heptane-2,3-dicarboxylic acid (endothall); pentachlorophenol (PCP); 2,6-dichlorobenzonitrile (dichlobenil). The actual formulations of these organic herbicides are listed in the discussion of each compound. The two inorganic herbicides tested were technical grades of sodium arsenite and copper sulfate pentahydrate.

RESULTS

Silvex

Silvex was tested as a liquid formulation containing 47 percent of the butoxy ethanol ester (BEE) as the active ingredient. Five species of fish were used in extensive testing in the two diluent waters. Laurel No. 1 diluent was used in five series with bluegills, five series with bluespotted sunfish, and one series with black bullhead. Laurel No. 2 diluent was used in two series with bluegills, three series with rainbow trout, and one series with goldfish. Average total lengths, weights, and loading levels were as follows: rainbow trout, 46 mm, 0.67 gm, 0.45 gm/1; goldfish, 41 mm, 1.25 gm, 0.83 gm/1; black bullhead, 125 mm, 15 gm, 2.0 gm/1; bluespotted sunfish, 37 mm, 0.70 gm, 0.47 gm/1; bluegills, 40 mm, 0.75 gm, 0.50 gm/1.

The acute toxicity values, derived from the mortality data, are presented in tables 3-5. Although all bioassays were run over a 96-hour period, the LC50 for the 72-hour and 96-hour observations showed no change from the 48-hour values with one exception. A slight lowering of the 72-hour

Table 1:--Chemical characteristics of Laurel #1 diluent at four levels of hardness.

Characteristic	Hardness Classification			
	Very soft	Soft	Medium	Hard
Calcium (ppm)	1.93	7.75	31.00	54.27
Magnesium (ppm)	1.68	6.73	26.91	47.09
Sodium (ppm)	18.25	18.25	18.25	18.25
Potassium (ppm)	1.05	1.05	1.05	1.05
Sulfate (ppm)	11.25	45.15	180.60	316.06
Bicarbonate (ppm)	48.45	48.45	48.45	48.45
Chloride (ppm)	0.95	0.95	0.95	0.95
M. O. Alkalinity (ppm as $CaCO_3$)	48.0	48.0	48.0	48.0
Total Hardness (ppm as $CaCO_3$)	13.0	52.2	208.7	365.2
Specific Conductance (μmohs, 25° C.)	135.0	195.0	415.0	650.0
pH	7.8±0.2	8.0±0.2	8.0±0.2	8.0±0.2

Table 2:--Chemical characteristics of Laurel #2 diluent at three levels of hardness.

Characteristic	Hardness Classification		
	Soft	Medium	Hard
Calcium (ppm)	13.46	53.82	94.19
Magnesium (ppm)	2.46	9.86	17.25
Sodium (ppm)	7.61	7.61	7.61
Potassium (ppm)	1.15	1.15	1.15
Sulfate (ppm)	41.99	167.93	293.88
Bicarbonate (ppm)	20.19	20.19	20.19
Chloride (ppm)	1.05	1.05	1.05
M. O. Alkalinity (ppm as $CaCO_3$)	25.0	25.0	25.0
Total Hardness (ppm as $CaCO_3$)	52.5	210.0	368.4
Specific Conductance (μmohs, 25° C.)	140.0	400.0	600.0
pH	7.3±0.2	7.5±0.2	7.7±0.2

LC50 from the 48-hour value was recorded in all bioassays with bluespotted sunfish (table 4). This lack of change between the 48-hour LC50 and both the 72-hour and 96-hour values was apparent in nearly all bioassays using phenoxy compounds, and is discussed below.

Where two or more sets of data were available, the LC50 data from the bioassays with bluegills (tables 3a and 3b), bluespotted sunfish (table 4), and rainbow trout (table 5) were each subjected to analyses of variance. No significant treatment differences were demonstrated among hardness levels or between the two types of diluent. In all but one case, the calculated F values were less than unity. The F value of 1.440 (2 and 3 d.f.), computed for the 12-hour rainbow trout data, lacked significance at the 95 percent level.

Acute toxicity values in the single series with goldfish (table 5) were approximately

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Table 3a:--Acute toxicity in ppm of silvex (BEE) to bluegills at four water hardnesses in Laurel #1 diluent.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC_{50} (95% Conf. limits)	12 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)
I	300	Soft	—	0.62(0.58-0.66)	0.57(0.54-0.60)	0.52(0.49-0.55)
		Medium	—	0.70(0.66-0.76)	0.54(0.50-0.58)	0.53(0.50-0.56)
		Hard	—	0.64(0.61-0.68)	0.55(0.52-0.58)	0.51(0.47-0.55)
II	400	Very Soft	—	0.79(0.66-0.94)	0.46(0.43-0.49)	0.42(0.40-0.44)
		Soft	—	0.76(0.63-0.90)	0.43(0.40-0.46)	0.40(0.37-0.43)
		Medium	—	0.72(0.63-0.83)	0.47(0.44-0.50)	0.43(0.41-0.46)
		Hard	—	0.70(0.62-0.78)	0.40(0.38-0.43)	0.37(0.35-0.39)
III	400	Very Soft	1.52(1.33-1.73)	—	—	—
		Soft	1.25(1.11-1.41)	0.62(0.56-0.69)	0.47(0.43-0.51)	—
		Medium	1.18(1.09-1.28)	0.62(0.58-0.68)	0.46(0.42-0.50)	—
		Hard	1.13(1.02-1.25)	0.62(0.56-0.68)	0.41(0.36-0.46)	—
IV	300	Soft	—	—	0.48(0.46-0.51)	0.40(0.37-0.44)
		Medium	—	—	0.50(0.47-0.53)	0.49(0.45-0.53)
		Hard	—	—	0.43(0.40-0.46)	0.38(0.36-0.41)
V	720	Very Soft	1.04(0.97-1.11)	0.64(0.60-0.68)	0.44(0.42-0.47)	0.38(0.36-0.41)
		Soft	0.87(0.79-0.95)	0.58(0.54-0.64)	0.44(0.41-0.47)	0.42(0.39-0.45)
		Hard	1.00(0.91-1.10)	0.58(0.53-0.63)	0.44(0.41-0.47)	0.40(0.37-0.43)
Average LC_{50} Laurel #1		Very Soft	1.28	0.72	0.45	0.40
		Soft	1.06	0.64	0.48	0.44
		Medium	1.18[2/]	0.68	0.49	0.48
		Hard	1.06	0.64	0.45	0.42

1/ See Table 1 for total hardness values.
2/ Single value only.

Table 3b:--Acute toxicity in ppm of silvex (BEE) to bluegills at three water hardnesses in Laurel #2 diluent.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC_{50} (95% Conf. limits)	12 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)
VI	150	Soft	1.18(1.04-1.33)	0.68(0.56-0.83)	0.56(0.46-0.69)	0.51(0.42-0.62)
		Medium	0.92(0.78-1.08)	0.70(0.59-0.83)	0.54(0.45-0.63)	0.47(0.41-0.54)
		Hard	0.91(0.74-1.10)	0.56(0.46-0.69)	0.49(0.44-0.54)	—
VII	300	Soft	1.14(1.03-1.26)	—	0.43(0.39-0.49)	—
		Medium	1.12(1.01-1.24)	—	0.40(0.35-0.45)	—
		Hard	1.07(0.94-1.22)	—	0.40(0.35-0.45)	—
Average LC_{50} Laurel #2		Soft	1.16	—	0.50	—
		Medium	1.02	—	0.47	—
		Hard	0.99	—	0.44	—

1/ See Table 2 for total hardness values.

Table 4:--Acute toxicity in ppm of silvex (BEE) to bluespotted sunfish at four water hardnesses in Laurel #1 diluent.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC_{50} (95% Conf. limits)	12 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)
I	360	Soft	—	0.49(0.46-0.53)	0.42(0.39-0.45)	0.41(0.38-0.44)
		Medium	—	0.48(0.45-0.51)	0.46(0.44-0.48)	0.46(0.44-0.48)
		Hard	—	0.54(0.50-0.57)	0.49(0.47-0.51)	0.49(0.46-0.51)
II	240	Very Soft	—	0.58(0.52-0.64)	0.44(0.42-0.47)	0.44(0.42-0.47)
		Medium	—	0.58(0.53-0.64)	0.47(0.44-0.51)	0.45(0.42-0.49)
		Hard	—	0.59(0.54-0.64)	0.51(0.48-0.54)	0.50(0.47-0.53)
III	320	Very Soft	—	0.65(0.56-0.75)	0.51(0.46-0.56)	0.50(0.46-0.55)
		Soft.	—	0.74(0.68-0.79)	0.72(0.64-0.82)	0.72(0.64-0.82)
		Medium	—	0.84(0.71-0.99)	0.67(0.60-0.74)	0.63(0.58-0.69)
		Hard	—	0.62(0.57-0.68)	0.56(0.53-0.60)	0.54(0.50-0.58)
IV	80	Soft	—	0.64(0.59-0.68)	—	—
		Hard	—	0.52(0.47-0.58)	—	—
V	400	Very Soft	1.08(0.94-1.24)	0.76(0.66-0.88)	0.76(0.68-0.84)	0.72(0.62-0.83)
		Soft	1.14(0.96-1.24)	0.74(0.62-0.88)	0.70(0.60-0.82)	0.63(0.56-0.72)
		Medium	0.92(0.82-1.04)	0.60(0.55-0.66)	0.53(0.46-0.61)	0.50(0.43-0.58)
Average LC_{50} Laurel #1		Very Soft	—	0.66	0.57	0.55
		Soft	—	0.65	0.61	0.59
		Medium	—	0.62	0.53	0.51
		Hard	—	0.58	0.54	0.53

1/ See Table 1 for total hardness values.

equal for the three levels of hardness tested at the 6-, 12-, and 24-hour period. The 48-hour LC50 for this species, however, was measurably lower in both higher levels of hardness as compared to the LC50 in the soft water indicating a greater toxicity in soft water. Limited data for black bullheads indicated that the toxicity of silvex increased as the hardness increased. The data from both the goldfish and black bullhead bioassays were insufficient to test for significance. The differences in toxicity among the three levels of hardness as reported in table 5 for these two species may only be due to variations inherent with the use of small numbers of individuals and lack of replications to minimize such variations.

2, 4-D

Three liquid formulations of 2, 4-D were tested against rainbow trout, goldfish, black bullheads, and bluegills. Except for the goldfish, average lengths, weights and loading levels were the same as reported for the silvex bioassays. For tests with this herbicide, goldfish averaged 80 mm in total length, 1.0 gm in weight, and were used at a loading level of approximately 0.33 gm/1.

The most extensively tested formulation contained 62.5 percent of the butoxy ethanol ester (BEE) as the active ingredient. This formulation was tested against bluegills in eight test series using Laurel No. 1 diluent (table 6a) and two test series using Laurel No. 2 diluent (table 6b). The BEE formulation was also used in one test series using black bullheads in Laurel No. 1 diluent. Rainbow trout were tested in three series, and goldfish in one series, with Laurel No. 2 diluent and the butoxy ethanol ester. The toxicity data for the black bullheads, trout, and goldfish tested

Table 5:--Acute toxicity in ppm of silvex (BEE) to black bullhead, goldfish, and rainbow trout at three water hardnesses.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC50 (95% Conf. limits)	12 Hour LC50 (95% Conf. limits)	24 Hour LC50 (95% Conf. limits)	48 Hour LC50 (95% Conf. limits)
Black Bullhead Laurel #1	150	Very Soft	8.55(6.02-10.73)	—	4.20(3.34-5.44)	—
		Soft	6.20(5.08-7.56)	—	3.60(2.95-4.39)	—
		Hard	7.50(5.67-9.92)	—	1.90(1.22-2.81)	—
Goldfish Laurel #2	300	Soft	2.50(estimated)	1.40(1.21-1.62)	1.07(0.90-1.27)	0.95(0.81-1.11)
		Medium	2.66(2.22-3.18)	1.45(1.26-1.67)	0.87(0.74-1.01)	0.68(0.57-0.81)
		Hard	2.20(1.88-2.57)	1.16(1.00-1.35)	0.83(0.69-1.00)	0.65(0.53-0.80)
Rainbow Trout Laurel #2	50	Soft	—	—	0.78(0.60-1.01)	0.48(0.36-0.65)
	300	Soft	1.73(1.54-1.94)	0.75(0.66-0.86)	0.67(0.58-0.77)	—
		Medium	1.78(1.57-2.02)	0.78(0.66-0.91)	0.57(0.49-0.68)	—
		Hard	1.85(1.64-2.08)	0.75(0.65-0.85)	0.68(0.58-0.79)	—
	300	Soft	—	0.65(0.60-0.70)	0.58(0.48-0.70)	—
		Medium	—	0.76(0.71-0.82)	0.67(0.62-0.72)	—
		Hard	—	0.74(0.69-0.80)	0.67(0.63-0.72)	—
Average LC50 Laurel #2		Soft	—	0.70	0.68	—
		Medium	—	0.77	0.62	—
		Hard	—	0.74	0.68	—

1/ For total hardness values see Tables 1 and 2.

against this formulation are presented in table 7.

A 70 percent formulation of the propylene glycol butyl ether ester (PGBEE) was used in three series of tests against bluegills, two of which were run in Laurel No. 1 diluent while the third was run in Laurel No. 2 diluent. A single test series was run with a 49.5 percent formulation of the dimethylamine salt (DMS) as the active ingredient. This test, with bluegills, was run at only two levels of hardness in Laurel No. 2 diluent. The toxicity data for the PGBEE and the DMS formulations are presented in table 8.

The data obtained from the 2, 4-D BEE series with bluegill and rainbow trout as well as the PGBEE series with bluegill, were subjected to analyses of variance. No significant difference among levels of water hardness or between the two diluents was demonstrated. As with the silvex data, the calculated F values were all less than unity with two exceptions. The F values for the 12-hour and 24-hour bluegill-PGBEE data were 5.5025 (5 and 2 d.f.) and 1.3907 (5 and 3 d.f.) respectively and lacked significance at the 95 percent level.

The data resulting from the bioassays of 2, 4-D BEE with goldfish and black bullhead and the DMS series with bluegill, though insufficient for statistical treatment, indicate that water hardness has little or no effect.

Endothall

Two "coco" amine salts of endothall were tested, as liquid formulations, against goldfish, bluegills, and redear sunfish in Laurel No. 2 diluent. The di(N, N-dimethylalkylamine) salt was tested in two series each with goldfish and bluegills, and in only one series with redear sunfish (table 9). The mono (N, N-dimethylalkylamine) salt was tested in three series with

Table 6a:--Acute toxicity in ppm of 2,4-D (BEE) to bluegills at three water hardnesses in Laurel #1 diluent.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC50 (95% Conf. limits)	12 Hour LC50 (95% Conf. limits)	24 Hour LC50 (95% Conf. limits)	48 Hour LC50 (95% Conf. limits)
I	60	Soft	1.93(1.65-2.26)	—	1.07(0.90-1.27)	—
II	300	Soft	—	—	1.05(0.99-1.12)	1.02(0.96-1.08)
		Medium	—	—	1.18(1.11-1.26)	0.16(1.07-1.26)
		Hard	—	—	1.03(0.96-1.10)	0.96(0.84-1.10)
III	200	Soft	—	—	1.45(1.29-1.63)	1.38(1.27-1.50)
		Hard	—	—	1.58(1.43-1.75)	1.58(1.43-1.75)
IV	300	Soft	—	2.07(1.74-2.46)	1.97(1.89-2.05)	—
		Medium	—	2.51(2.12-2.97)	2.20(2.02-2.39)	—
		Hard	—	2.18(1.95-2.44)	1.70(1.57-1.84)	—
V	300	Soft	1.48(1.39-1.58)	—	—	—
		Medium	1.33(1.25-1.42)	—	—	—
		Hard	1.06(0.98-1.15)	—	—	—
VI	300	Soft	—	1.97(1.72-2.26)	1.41(1.32-1.51)	1.38(1.31-1.45)
		Medium	—	1.68(1.57-1.80)	1.41(1.32-1.51)	1.36(1.27-1.45)
		Hard	—	1.59(1.52-1.67)	1.38(1.28-1.49)	1.36(1.27-1.45)
VII	300	Soft	2.87(2.69-3.06)	1.96(1.84-2.09)	1.75(1.64-1.87)	1.73(1.61-1.86)
		Medium	2.98(2.81-3.16)	2.17(2.03-2.32)	2.05(1.94-2.16)	2.00(1.86-2.16)
		Hard	3.00(2.76-3.24)	2.28(2.14-2.42)	2.20(2.07-2.34)	2.10(1.97-2.24)
VIII	100	Hard	—	—	1.32(1.18-1.47)	1.33(1.16-1.52)
Average LC50 Laurel #1		Soft	2.09	2.00	1.46	1.38
		Medium	2.16	2.12	1.71	1.51
		Hard	2.03	2.02	1.54	1.47

1/ See Table 1 for total hardness values.

Table 6b:--Acute toxicity in ppm of 2,4-D (BEE) to bluegills at three water hardnesses in Laurel #2 diluent.

Series	No. of fish/ series	Water[1/] hardness	6 Hour LC50 (95% Conf. limits)	12 Hour LC50 (95% Conf. limits)	24 Hour LC50 (95% Conf. limits)	48 Hour LC50 (95% Conf. limits)
VII	150	Soft	2.19(1.95-2.46)	—	1.38(1.16-1.64)	—
		Medium	2.40(2.14-2.69)	—	1.46(1.35-1.57)	—
		Hard	2.60(2.34-2.88)	—	1.78(1.60-1.96)	—
VIII	300	Soft	2.80(2.44-3.22)	—	2.10(1.81-2.43)	—
		Medium	2.60(2.36-2.86)	—	1.90(1.74-2.08)	—
		Hard	2.70(2.42-3.02)	—	2.18(1.94-2.45)	—
Average LC50 Laurel #2		Soft	2.50	—	1.74	—
		Medium	2.50	—	1.68	—
		Hard	2.65	—	1.98	—

1/ See Table 2 for total hardness values.

Table 7:--Acute toxicity in ppm of 2,4-D (BEE) to black bullhead, goldfish, and rainbow trout at three water hardnesses.

Species & diluent	No. of fish/ series	Water 1/ hardness	6 Hour LC_{50} (95% Conf. limits)	12 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)
Black Bullhead Laurel #1	150	Soft	11.20(9.69-12.95)	—	8.70(7.87-9.62)	7.70(7.10-8.35)
		Medium	8.90(6.95-11.39)	—		
		Hard	10.60(9.36-12.00)	—	8.80(7.75-9.99)	7.10(6.40-7.88)
Goldfish Laurel #2	300	Soft	5.78(4.90-6.82)	4.55(3.98-5.20)	4.10(3.56-4.72)	2.65(2.24-3.14)
		Medium	5.00(4.40-5.68)	3.95(3.33-4.68)	3.63(3.13-4.20)	2.72(2.25-3.29)
		Hard	5.17(4.46-6.00)	4.50(4.01-5.05)	3.98(3.43-4.62)	3.62(2.18-3.14)
Rainbow Trout Laurel #2	50	Soft			1.70(1.26-2.29)	1.50(1.15-1.97)
	300	Soft	2.81(2.41-3.27)	1.99(1.80-2.21)	1.49(1.32-1.68)	1.42(1.27-1.59)
		Medium	3.44(3.12-3.79)	2.30(2.84-2.59)	1.83(1.66-2.02)	1.55(1.41-1.70)
		Hard	3.20(2.90-3.54)	2.03(1.84-2.24)	1.67(1.53-1.83)	1.50(1.37-1.64)
	300	Soft	—	1.90(1.71-2.11)	1.50(1.40-1.61)	—
		Medium	—	1.61(1.43-1.81)	1.28(1.08-1.52)	—
		Hard	—	1.93(1.72-2.16)	1.38(1.30-1.46)	—
Average LC_{50} Laurel #2		Soft	—	1.94	1.56	1.46
		Medium	—	1.96	1.56	—
		Hard	—	1.98	1.52	—

1/ For total hardness values see Tables 1 and 2.

Table 8:--Acute toxicity in ppm of 2,4-D (PGBEE) and (DMS) to bluegills at three water hardnesses.

Formulation & diluent	Series & No. of fish series	Water 1/ Hardness	6 Hour LC_{50} (95% Conf. limits)	12 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf limits)	48 Hour LC_{50} (95% Conf. limits)
PGBEE Laurel #1	I 360	Soft	—		1 43(1.30-1.57)	1 18(1.09-1.28)
		Medium	—	1.49(1.33-1 66)	1.14(1.07-1.21)	1.03(0.94-1.13)
		Hard	—	1.60(1.38-1.85)	1.20(1.11-1.30)	0.99(0.92-1.07)
PGBEE Laurel #1	II 150	Soft	1.53(1.22-1.70)	1.28(1.05-1.56)	1.14(0.98-1.33)	—
		Medium	1.76(1.47-2.11)	1.38(1.15-1.65)	1.00(0.76-1.29)	—
		Hard	2.02(1.76-2.32)	1.49(1.29-1.72)	1.19(1.03-1.38)	—
PGBEE Laurel #2	III 300	Soft	2.42(2.20-2.66)	1.28(1.16-1.41)	1.02(0.93-1.12)	—
		Medium	2.41(2.18-2.67)	1.14(0.97-1.33)	0.93(0.83-1.04)	—
		Hard	2.25(2.04-2.48)	1.20(1.07-1.36)	1.06(0.96-1.17)	—
PGBEE Average LC_{50} all series		Soft	1.96	1.28	1.20	—
		Medium	2.08	1.34	1.02	—
		Hard	2.14	1.43	1.15	—
DMS Laurel #2	I 200	Soft	290(257-327)	—	193(175-213)	144(estimated)
		Medium	282(246-323)	—	176(154-201)	140(114-172)

1/ For total hardness values see Tables 1 and 2.

Table 9:--Acute toxicity in ppm of di (N,N-dimethylalkylamine) salt of endothall to goldfish, bluegills, and redear sunfish at three water hardnesses in Laurel #2 diluent.

Species & series	No. of fish/ series	Water 1/ hardness	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)	72 Hour LC_{50} (95% Conf. limits)
Goldfish I	300	Soft	0.86(0.76-0.98)	0.80(0.73-0.88)	—
		Medium	0.82(0.14-0.91)	0.80(0.73-0.88)	—
		Hard	1.17(1.03-1.33)	1.03(0.90-1.18)	—
II	300	Soft	1.00(0.90-1.12)	0.86(0.81-0.92)	0.86(0.81-0.92)
		Medium	1.19(1.10-1.28)	0.97(0.87-1.08)	0.97(0.87-1.08)
		Hard	1.26(1.13-1.40)	1.15(1.06-1.25)	1.15(1.06-1.25)
Bluegill I	300	Soft	0.91(0.79-1.05)	0.63(0.56-0.71)	0.60(0.54-0.65)
		Medium	0.78(0.72-0.84)	0.68(0.62-0.75)	0.54(0.50-0.59)
		Hard	1.02(0.95-1.10)	0.80(0.72-0.88)	0.72(0.66-0.78)
II	300	Soft	0.84(0.76-0.92)	0.78(0.71-0.87)	0.78(0.71-0.87)
		Medium	0.88(0.81-0.96)	0.84(0.77-0.91)	0.84(0.77-0.91)
		Hard	0.87(0.79-0.95)	0.87(0.78-0.96)	0.87(0.78-0.96)
Redear I	300	Soft	0.53(0.47-0.60)	—	—
		Medium	0.56(0.52-0.62)	—	—
		Hard	0.56(0.50-0.62)	—	—

1/ See Table 2 for hardness values.

goldfish, two series with bluegills, and one series with redear sunfish (table 10). Average total lengths, weights, and loading levels for the bioassays of both "coco" amine salts were: goldfish, 54.5 mm, 1.95 gm, 0.87 gm/l; bluegill, 38.0 mm, 0.71 gm, 0.47 gm/l; redear sunfish, 53.0 mm, 1.20 gm, 0.80 gm/l.

A single bioassay series was run with a 19.2 percent liquid formulation of the disodium salt of endothall. This series was run with bluegill which averaged 33.0 mm in total length and 0.78 gm in weight, resulting in a loading level of 0.52 gm/l. The results of this series are shown in table 11.

The data from the bioassays of both alkylamine salts of endothall with goldfish and bluegills were subjected to analyses of variance. No significant effect of water hardness on either of these salts was demonstrated. The maximum F value (2 and 3 d.f.) recorded in these analyses was 1.6 which lacked significance at the 95 percent level. Inspection of the limited data from the bioassays of the disodium salt indicates no effect attributable to water hardness.

Pentachlorophenol

A formulation of 8 percent pentachlorophenol in an oil base was used in two series each with goldfish and bluegill in Laurel No. 2 diluent. The average total length, weight, and loading levels were: goldfish, 58.2 mm, 2.6 gm, 0.87 gm/l; bluegill, 38.8 mm, 0.51 gm, 0.34 gm/l. Results of these test series are tabulated in table 12. In the first series with goldfish in which five concentrations (ranging from 0.024 to 0.042 ppm) were used, the only kill occurred in the 96-hour soft water series, and consequently this is the only value shown for this test series in table 12.

The 24-hour and 72-hour bluegill data were subjected to analyses of variance. No effect of water hardness was demonstrated. The F values for these two sets

Table 10:--Acute toxicity in ppm of the mono (N,N-dimethylalkylamine) salt of endothall to goldfish, bluegills, and redear sunfish at three water hardnesses in Laurel #2 diluent.

Species & series	No. of fish/ series	Water[1/] hardness	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)	72 Hour LC_{50} (95% Conf. limits)	96 Hour LC_{50} (95% Conf. limits)
Goldfish I	300	Soft	0.81(0.74-0.88)	0.78(0.68-0.89)	—	—
		Medium	0.98(0.85-1.13)	0.95(0.85-1.06)	—	—
		Hard	1.17(1.04-1.21)	1.05(0.93-1.18)	—	—
II	300	Soft	—	1.50(1.35-1.66)	—	—
		Medium	—	1.38(1.28-1.49)	—	—
		Hard	—	1.52(1.38-1.67)	—	—
III	300	Soft	1.39(1.32-1.47)	1.23(1.16-1.31)	—	1.20(1.13-1.28)
		Medium	1.28(1.20-1.36)	1.18(1.11-1.26)	—	1.11(1.06-1.16)
		Hard	1.18(1.09-1.28)	1.14(1.08-1.21)	—	1.08(1.02-1.14)
Bluegill I	300	Soft	1.49(1.21-1.83)	1.34(1.17-1.53)	1.11(1.01-1.22)	—
		Medium	1.02(0.94-1.10)	0.94(0.90-0.98)	0.90(0.86-0.95)	—
		Hard	1.12(1.06-1.19)	1.03(0.95-1.11)	1.02(0.96-1.09)	—
II	300	Soft	1.09(1.01-1.18)	1.02(0.96-1.09)	1.01(0.91-1.12)	—
		Medium	1.02(0.94-1.11)	1.00(0.92-1.08)	1.00(0.92-1.08)	—
		Hard	0.90(0.85-0.95)	0.90(0.85-0.95)	0.90(0.85-0.95)	—
Redear I	300	Soft	0.81(0.78-0.84)	—	—	—
		Medium	0.68(0.65-0.72)	—	—	—
		Hard	0.73(0.69-0.80)	—	—	—

1/ See Table 2 for hardness values.

Table 11:--Acute toxicity in ppm of the disodium salt of endothall to bluegills at three water hardnesses in Laurel #1 diluent.

No. of fish/ series	Water[1/] hardness	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)	72 Hour LC_{50} (95% Conf. limits)	96 Hour LC_{50} (95% Conf. limits)
300	Soft	277(234-328)	219(198-242)	179(165-194)	140(126-156)
	Medium	249(222-279)	181(166-197)	123(108-140)	105(88-125)
	Hard	280(242-324)	196(178-215)	133(119-148)	102(84-124)

1/ See Table 1 for hardness values.

of analyses were 1.500 (2 and 3 d.f.) and 0.0333 (2 and 3 d.f.) respectively.

Dichlobenil

A technical formulation of dichlobenil containing 96.4 percent active ingredient was tested in two series with bluegill. The fish averaged 30.8 mm in total length and 0.56 gm in weight and were used at a loading level of 0.37 gm/l. Tests were run in Laurel No. 2 diluent only. The data from these tests are presented in table 13.

The data from these bioassays were subjected to analyses of variance for all five time periods reported in table 13. In all cases the F values were less than unity, indicating no significant effect of water hardness on this herbicide.

Table 12:--Acute toxicity in ppm of pentachlorophenol to goldfish and bluegills at three water hardnesses in Laurel #2 diluent.

Species series & no./series	Water[1/] hardness	6 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)	72 Hour LC_{50} (95% Conf. limits)	96 Hour LC_{50} (95% Conf. limits)
Goldfish I 50	Soft	—	—	—	—	.023(.020-.026)
Goldfish II 300	Soft	—	—	.169(.112-.255)	.084(.058-.123)	.056(.042-.077)
	Medium	—	—	.081(.069-.095)	.072(.061-.084)	.055(.048-.063)
	Hard	—	—	.108(.084-.138)	.057(.047-.070)	.049(.039-.061)
Bluegill I 150	Soft	—	.038(.035-.042)	—	.025(.022-.029)	—
	Medium	—	.045(.038-.054)	—	.026(.022-.030)	—
	Hard	—	.053(.042-.068)	—	.027(.023-.031)	.024(.020-.028)
Bluegill II 300	Soft	.083(.069-.100)	.054(.047-.060)	.033(.030-.037)	.030(.027-.034)	—
	Medium	.057(.051-.063)	.039(.034-.044)	.029(.025-.033)	.025(.022-.029)	—
	Hard	.052(.047-.057)	.046(.041-.050)	.039(.035-.043)	.029(.026-.032)	—

1/ See Table 2 for total hardness values.

Table 13:--Acute toxicity in ppm of dichlobenil to bluegills at three water hardnesses in Laurel #2 diluent.

Series & no. fish/ series	Water[1/] hardness	6 Hour LC_{50} (95% Conf. limits)	24 Hour LC_{50} (95% Conf. limits)	48 Hour LC_{50} (95% Conf. limits)	72 Hour LC_{50} (95% Conf. limits)	96 Hour LC_{50} (95% Conf. limits)
I 300	Soft	22.2(19.7-25.1)	18.2(16.5-20.0)	17.3(15.4-19.4)	15.0(13.3-16.9)	13.3(11.1-15.9)
	Medium	21.5(19.0-24.3)	16.8(14.9-18.9)	15.4(13.7-17.3)	14.0(12.5-15.6)	11.0(9.2-13.2)
	Hard	20.2(18.3-22.2)	18.0(15.7-20.6)	17.5(15.9-19.3)	14.5(13.2-16.0)	11.0(9.5-12.8)
II 300	Soft	20.4(17.9-23.3)	18.2(16.3-20.3)	14.4(12.7-16.3)	13.3(11.9-14.9)	8.8(7.2-10.8)
	Medium	22.0(19.3-25.1)	21.4(18.5-24.8)	16.9(14.2-20.1)	13.0(11.1-15.2)	9.0(7.4-10.9)
	Hard	24.0(21.7-26.5)	21.2(19.0-23.6)	16.3(14.3-18.5)	14.6(12.8-16.7)	10.0(8.2-12.2)
Average LC_{50}	Soft	21.3	18.2	15.8	14.2	11.0
	Medium	21.8	19.1	16.2	13.5	10.0
	Hard	22.1	19.6	16.9	14.6	10.5

1/ See Table 2 for total hardness values.

Sodium arsenite

Sodium arsenite was tested as the technical grade material in two series each in Laurel No. 1 and No. 2 diluents. The only species tested was bluegill which averaged 44.0 mm in total length and 0.80 gm in weight and was used at an average loading level of 0.53 gm/l. The results of these tests are shown in table 14.

These data were subjected to analyses of variance both between diluent types and among the three levels of hardness. No significant effect was demonstrated between types of diluent or among hardness levels.

Copper sulfate

Copper sulfate was tested as the reagent grade of the pentahydrated form (blue vitriol) in one series with Laurel No. 1

Table 14:--Acute toxicity in ppm ($NaAsO_2$) of sodium arsenite to bluegills at three water hardnesses in two types of diluent waters.

Diluent, series & no./series	Water hardness [1]	6 Hour	24 Hour	48 Hour	72 Hour	96 Hour
		LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits
Laurel #1	Soft	___	59.0(35.8-68.7)	41.0(36.4-46.3)	36.4(24.6-53.9)	___
I	Medium	___	61.0(52.2-71.3)	39.0(32.5-46.8)	30.7(23.6-39.6)	___
300	Hard	___	52.7(45.4-61.1)	38.0(32.8-44.0)	30.7(26.2-35.6)	___
Laurel #1	Soft	133(110-161)	57.5(48.7-67.8)	48.1(40.0-57.6)	36.6(31.6-42.5)	24.0(19.8-29.4)
II	Medium	150(115-175)	78.5(65.4-94.2)	52.9(43.7-64.0)	39.0(32.6-46.8)	32.7(27.5-38.9)
300	Hard	118(97-143)	83.0(69.2-99.6)	58.5(49.2-69.6)	37.2(31.9-43.4)	29.8(24.8-35.8)
Laurel #2	Soft	___	___	50.4(32.3-78.6)	38.5(24.5-60.4)	29.0(18.8-44.7)
III	Medium	___	___	46.2(29.6-72.1)	31.5(22.0-45.2)	23.6(17.5-31.7)
300	Hard	___	___	50.8(30.6-84.3)	36.4(25.5-52.1)	23.6(17.5-31.7)
Laurel #2	Soft	138(112-171)	54.3(46.7-63.2)	46.1(38.9-54.6)	34.0(24.9-46.4)	___
IV	Medium	155(112-215)	52.5(45.1-61.1)	38.7(31.0-48.4)	___	___
300	Hard	144(109-190)	51.3(44.0-59.8)	36.0(27.3-47.5)	___	___
Average	Soft	136	56.9	46.4	36.4	26.5
LC_{50}	Medium	152	64.0	44.2	33.4	28.0
All series	Hard	131	62.3	45.8	34.8	26.7

[1] For total hardness values see Tables 1 and 2.

diluent and two series with Laurel No. 2 diluent. Bluegills averaging 42.0 mm in total length, 0.59 gm in weight, were tested at a loading level of 0.39 gm/l. The results of these three series of tests are presented in table 15.

In all series and at all observation times, there was a noticeable reduction in the toxicity of copper sulfate with an increase of hardness. The data shown in table 15 were subjected to analyses of variance and in all cases the differences among the three levels of hardness were significant. In order to obtain LC50 values in the medium and hard waters, the concentration of copper sulfate had to be increased by approximately 35 percent and 100 percent, respectively, over that used in the soft water. The effect of the calcium/magnesium ratio seemed less important than the effect of hardness itself, but the amount of data were insufficient to test this hypothesis.

DISCUSSION

The results of this investigation indicate that water hardness, within the range found in fish-bearing waters of the United States, has no significant effect on the toxicity to several species of fish for the following eight herbicides: silvex (BEE); 2,4-D (BEE); 2,4-D (PGBEE); di(N, N-dimethylalkylamine) salt of endothall; mono (N, N-dimethylalkylamine) salt of endothall; PCP; dichlobenil; sodium arsenite. Limited data also indicate no probable effect of water hardness on the fish-toxicity of 2,4-D (DMS) and disodium endothall. Copper sulfate was the only herbicide tested which showed a definite effect of water hardness on its toxicity to bluegill sunfish. The average 24-hour, 48-hour, and 96-hour LC50 values at three levels of water hardness for bluegills, for all 11 herbicides, are summarized in table 16.

Several explanations of variations between test replications in this study, as

Table 15:--Acute toxicity in ppm ($CuSO_4$) of copper sulfate to bluegills at three water hardnesses in two types of diluent waters.

Series & Diluent	No. of fish/series	Water hardness [1]	6 Hour	24 Hour	48 Hour	72 Hour	96 Hour
			LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits	LC_{50} 95% Conf. limits
I		Soft	3.68(3.12-4.34)	2.10(1.82-2.43)	1.68(1.37-2.07)	1.08(0.82-1.44)	1.00(0.75-1.33)
Laurel	420	Medium	4.36(3.75-5.07)	3.24(2.84-3.69)	2.66(2.28-3.10)	1.85(1.65-2.07)	1.72(1.53-1.93)
#1		Hard	8.05(6.47-10.02)	3.70(3.16-4.33)	3.00(2.53-3.56)	2.68(2.35-3.06)	2.55(1.81-2.91)
II		Soft	3.48(3.02-4.01)	2.80(2.41-3.26)	2.26(1.82-2.81)	1.58 estimated	___
Laurel	300	Medium	4.34(3.78-5.08)	2.32(1.89-2.85)	2.16(1.61-2.89)	2.16(1.61-2.89)	___
#2		Hard	8.50(7.00-10.33)	4.90(4.22-5.69)	4.50(3.89-5.21)	4.18(3.67-4.77)	___
III		Soft	3.66(2.78-4.81)	1.78(1.46-2.17)	1.58(1.29-1.94)	1.58(1.29-1.94)	___
Laurel	300	Medium	4.95(4.17-5.88)	3.25(2.88-3.66)	2.95(2.60-3.35)	2.72(2.41-3.06)	___
#2		Hard	8.90(6.98-11.35)	3.85(3.36-4.41)	3.53(3.00-4.15)	3.39(2.88-1.44)	___
Average		Soft	3.61	2.23	1.84	1.41	___
LC_{50}		Medium	4.88	2.94	2.59	2.24	___
All series		Hard	8.48	4.15	3.68	3.42	___

[1] For total hardness values see Tables 1 and 2.

Table 16:--Average acute toxicity in ppm of eleven herbicides to bluegills at three generalized water hardnesses.

Herbicide	24 Hour			48 Hour			96 Hour		
	Soft	Medium	Hard	Soft	Medium	Hard	Soft	Medium	Hard
silvex (BEE)	0.48	0.48	0.45	0.45	0.48	0.42	___	___	___
2,4-D (BEE)	1.52	1.70	1.65	1.38	1.84	1.47	___	___	___
2,4-D (PGBEE)	1.20	1.02	1.15	1.18[1]	1.03[1]	0.99[1]	___	___	___
2,4-D (DMS)	193[1]	176[1]	___	144[1]	140[1]	___	___	___	___
di alkylamine endothall	0.88	0.83	0.94	0.70	0.76	0.83	___	___	___
mono alkylamine endothall	1.29	1.02	1.01	1.18	0.97	0.96	___	___	___
disodium endothall	277[1]	249[1]	280[1]	219[1]	181[1]	196[1]	140[1]	105[1]	102[1]
PCP	0.046	0.042	0.050	0.033[1]	0.029[1]	0.039[1]	___	___	0.024[1]
dichlobenil	18.2	19.1	19.6	15.8	16.2	16.9	11.0	10.0	10.5
sodium arsenite[2]	56.9	64.0	62.3	46.4	44.2	45.8	26.5	28.0	26.7
copper sulfate[3]	2.23	2.94	4.15	1.84	2.59	3.68	1.00[1]	1.72[1]	2.55[1]

[1] Values based on single observations.
[2] Concentrations expressed as ppm of $NaAsO_2$.
[3] Concentrations expressed as ppm of $CuSO_4$.

well as those ascribed to the effects of water hardness in the literature, can be made. The most apparent explanation arises from the differences in response by the test species themselves. Many authors have cited the different responses between two or more species exposed to the same chemical. In the present study, the extensive series of tests of the butoxy ethanol esters of both silvex and 2, 4-D with bluegills illustrate the variation which can be expected within a single species of test animal. The 24-hour LC50 for bluegills in the seven silvex (BEE) tests showed a variation of approximately 30 percent at each level of hardness. The average variation of the 24-hour LC50 in the ten 2, 4-D (BEE) tests was 66.7 percent.

Another possible explanation of the various toxic concentrations ascribed to hardness is found in the work of Hughes and Davis (1963) on variations in toxicity of several phenoxy herbicides to bluegill sunfish. These authors reported considerable variations in toxicity of 21 formulations of 2, 4-D, 6 each of silvex and 2, 4, 5-T, and 3 of 2-(2, 4-DP). The variations applied to formulations of different companies and formulations from different batch lots of a single company, as well as between the various amine and ester formulations. Some of the variation reported was ascribed to the effects of additives, surfactants, and carrier oils included in the commercial formulations. Their 24-hour TLm (LC50) values for bluegills were from two to more than ten times as great as their lowest value reported. These variations are much greater than the differences ascribed to the effect of water hardness by other authors for similar herbicides (Davis and Hardcastle, 1959; Surber and Pickering, 1962; Tarzwell, 1963).

Another factor which is thought to affect the toxicity of herbicides is pH. Simon and Beevers (1951, 1952a, 1952b) reported that the concentrations of weak acids (such as 2, 4-D) needed to produce a standard response remains constant at pH levels below the pK value of the acid but increases with levels of pH above the pK value, indicating a loss of activity in the more alkaline solutions.

Actual studies on the effect of pH on the toxicities of the organic herbicides (or related compounds) used in the present investigation are few and primarily related to their phytotoxicities. Blackman and Robertson-Cuninghame (1963) showed a direct effect of pH on the toxicity of 2, 4-D acid to the aquatic weed Lemna minor. At a concentration of 40 ppm of 2, 4-D acid, growth rates of this plant were maximally depressed at pH 4.6 while at pH of 6.1 the same concentration produced a minimal depression of the growth rate. Truchelut and Williams (1960b) using silvex and 2.4-D [acids?] concluded that pH level had a very strong influence on the efficiency of phenoxy-type herbicides against aquatic weeds. Goodnight (1942) and Crandall and Goodnight (1959, 1962) reported an increase in the toxicity of sodium PCP to several species of fish with a decrease in the pH of the test media used. Studies of Lipschuetz and Cooper (1961) demonstrated that decreased pH levels increased the toxicity of 2-secondary-butyl-4, 6-dinitrophenol [sic] to rainbow trout and blacknose dace (Rhinichthys atratulus). In three previously mentioned fish toxicity studies reporting the effects of water hardness on various herbicides (Webb, 1959; Davis and Hardcastle, 1959; Surber and Pickering, 1962), pH values varied with hardness. Walker (1963) did not report pH values.

In a study of the fate of 2, 4-D and its derivatives in surface water, Aly and Faust (1962) determined the rate of photodecomposition of 2, 4-dichlorophenol as well as the sodium salt, isopropyl and butyl esters of 2, 4-D at pH levels of 4.0, 7.0, and 9.0. The rate of photodecomposition of these compounds was markedly

increased at higher pH levels. Rapid rates of photodecomposition were recorded at pH 7.0, with a 50 percent loss in 50 minutes for these compounds under conditions of high ultraviolet irradiation at a peak wave length of 253.7 mu. Bell (1956), Crosby and Tutass (1966), and Tutass (1966) indicate that both natural sunlight and artificial ultraviolet light will cause photodecomposition of 2, 4-D acid and Sodium 2, 4-D in aqueous solutions.

In our tests, pH levels at the beginning of the tests in Laurel No. 1 diluent were 8.0 at all hardness levels, and in Laurel No. 2 diluent the pH ranged from 7.3 in the soft water to 7.7 in the hard water. During the actual test periods, the laboratory was continuously lighted by three banks of "daylight" fluorescent lights. The combination of pH levels between 7.3 and 8.0 plus continuous illumination with fluorescent lights, which presumably produces significant amounts of ultraviolet irradiation, may explain the apparent loss of toxicity of the phenoxy herbicides and possibly the PCP.

In their investigations of the fate of 2, 4-D in surface waters Faust and Aly (1963) and Aly and Faust (1964) found that the solubility of the calcium and magnesium salts of 2, 4-D was 4000 mg/1 and 11, 100 mg/1., respectively, and concluded that the ordinary amounts of calcium and magnesium ions in surface water would not remove 2, 4-D through precipitation reactions.

No explanation for the reduced toxicity of the two alkylamine salts of endothall beyond the 48-hour observation can be suggested. Hiltibran (1962) reported on the rapid disappearance of disodium endothall in field applications and concluded that the rate of disappearance depended on the presence of organisms and organic materials in the water. Walker (1963) reported rapid degradation in 1 to 4 days for the di(N, N-dimethylalkylamine) salt of endothall in aquarium tests.

Published information on the acute toxicity of dichlobenil to fish is scarce. Hughes and Davis (1962) reported on tests of several herbicides, including a wettable powder and granular formulation of dichlobenil, in water with a total hardness of 29 ppm. Their 24-hour TLM values for the two formulations were 17 and 37 ppm respectively, and their 48-hour TLM values were 17 and 30 ppm. Walker (1964) found 96-hour TLM values for several formulations of dichlobenil, including a technical material, to range from 10 to 20 ppm for four centrarchid fishes. His water quality table indicates a total hardness of about 55 ppm. In our tests we found that concentrations of the technical material of 32 and 42 ppm, in acetone, precipitated out immediately upon addition to the test containers 1/ and the precipitate remained throughout the four-day test. No precipitate was formed at the next lower concentration of 24 ppm. The nature of the precipitate was not determined.

The only references to the effect of water hardness on sodium arsenite relate to its efficacy in control of aquatic plants. Surber (1931) stated that water hardness would affect the amount of this herbicide needed to control aquatic vegetation. In a later publication (Surber, 1961), he indicated that 4 ppm would be equally effective against submersed aquatic weeds in both hard and soft waters. For discussions and references on the toxic concentrations of sodium arsenite to various species of fish the reader is referred to McKee and Wolf (1963).

The LC50 values for copper sulfate reported in tables 15 and 16 of this report are expressed as mg/1 (ppm) of the salt, $CuSO_4$. The actual toxicity of this soluble salt is due to the cupric ion in the water, and hence the correct concentration of the

1/ Information supplied by the manufacturer gave the solubility in water as 25 gm/1 at 25° C. Hughes and Davis (1962) indicated a solubility in water of 10 ppm at 25° C.

cupric ion in this study would be found by multiplying by a factor of 0.39812. The actual copper ion concentration for the average values presented in table 16, for the soft, medium, and hard waters respectively, would be as follows: 24-hour, 0.89 ppm, 1.17 ppm, 1.65 ppm; 48-hour, 0.73 ppm, 1.03 ppm, 1.46 ppm; 96-hour, 0.40 ppm, 0.68 ppm, 1.02 ppm.

The literature reviews of Doudoroff and Katz (1953) and McKee and Wolf (1963) cite numerous studies and reports of the toxicity of copper ions and/or copper sulfate. The toxicity values for copper sulfate from the present study are within the ranges reported for this compound. The effect of increased hardness on reducing the toxicity of this compound is verified for bluegill. No difference appeared to exist between the effects of the two diluents, Laurel No. 1 and Laurel No. 2, with Ca/Mg of ratios of approximately 1:1 and 5:1 respectively.

Our preliminary range-finding tests with copper sulfate confirmed observations of Carpenter (1930), Jones (1938), and Lloyd (1961) on the effects of size of fish, volume, and concentrations of the test solution on the toxicity of heavy metals. In one preliminary test with soft water (52.5 ppm), five of five bluegill were killed in 6 hours at a concentration of 1.56 ppm in 15 liters; in another test, 9 fish out of 10 of the same size survived the same conditions, and there appeared to be less precipitated mucus.

CONCLUSION

On the basis of the data obtained in this study, and results of earlier studies by other researchers, it is concluded that water hardness, as defined, has no significant effect on the toxicity to fish of the following eight herbicides: silvex (BEE); 2, 4-D (BEE); 2, 4-D (PGBEE); endothall di (N, N-dimethylalkylamine); endothall mono (N, N-dimethylalkylamine); pentachlorophenol; dichlobenil; sodium arsenite. Water hardness probably also has no significant effect on the fish-toxicity of 2, 4-D (DMS) and disodium endothall. As expected, increased calcium and magnesium hardness has a significant effect on the reduction of toxicity of copper sulfate to bluegill sunfish.

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