The Effects of Temperature on the Susceptibility of Bluegills and Rainbow Trout to Selected Pesticides

by KENNETH J. MACEK, CURT HUTCHINSON and OLIVER B. COPE
Fish-Pesticide Research Laboratory
U. S. Bureau of Sport Fisheries and Wildlife
Columbia, Missouri

Introduction

Consideration of interaction with environmental factors should constitute an important part of the evaluation of hazards posed to fish by pesticides. Temperatures in the aquatic environment under which exposure to pesticides occurs may vary considerably from one location to another, or over a period of time in the same habitat. Several reports have suggested that temperature can have a marked effect on the susceptibility of fish to pesticides (1, 2, 3). This report presents the results of an extensive investigation of the effects of temperature on the susceptibility of rainbow trout (*Salmo gairdneri* Richardson) and bluegills (*Lepomis macrochirus* Rafinesque) to a variety of pesticides.

Methods and Materials

The tests were conducted over a period of five

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 3, 1969, published by Springer-Verlag New York Inc.

years at the Fish-Pesticide Research Laboratory in Denver, Colo. and Columbia, Mo. The susceptibility of the fish to pesticides was measured in terms of the median tolerance limit (TL_{50}), which is defined as the concentration of the pesticide in water which causes 50 per cent mortality among the test fish under the test conditions. A modification of the method proposed by Litchfield and Wilcoxon (4) was used to calculate the median tolerance limit and its 95% confidence interval. Rather than visually fitting a line to the data plotted on log-probability graph paper as proposed by these authors, the concentrations tested and the observed per cent mortalities were converted to logs and probits, respectively, and a linear regression equation was calculated. The median tolerance limits were calculated for 24 and 96 hours of exposure at 1.6°, 7.2° and 12.7°C for rainbow trout, and at 12.7°, 18.3° and 23.8°C for bluegills.

Bioassays were simultaneously conducted at three temperatures in glass jars containing 15 liters of reconstituted water having a concentration of 2.0 mg/L of potassium chloride, 30.0 mg/L of magnesium sulfate, 30.0 mg/L of calcium sulfate, and 48.0 mg/L of sodium bicarbonate. The pH of the test water was 7.1 and the methyl orange alkalinity was 35 ppm. Technical grades of all compounds (Table 1) were used and a fresh stock solution of each pesticide was prepared in acetone the day of testing. Since test solutions were not renewed during the test period, the concentrations reported are those at the start of exposure. Groups of test fish ranged in average size from 0.6 to 1.5 grams and were obtained from National Fish Hatcheries. The fish used in testing any one compound were from the same lot and of the same weight and length (± 20%). Fish were conditioned to the test water and temperature for at least 48 hours prior to testing; during this period they were not fed. Test temperatures (± 0.6°C) were maintained by water baths. Twenty fish were tested at each concentration (ten per jar), and the mass/volume ratio never exceeded 1.0 gram of fish per liter of test solution. The control fish were exposed to the highest concentration of the solvent to which fish in the other test solutions were exposed.

Results

The median tolerance limit, and the relative increase in susceptibility[1/] of the test fish to the toxicant over the range of temperatures tested are presented

---

[1/] The relative increase in susceptibility is the ratio of the TL_{50} at the lowest temperature tested to the TL_{50} at the highest temperature tested.

TABLE 1

Common and chemical names of pesticides used in time-temperature tests

Common Name (Trade Name)	Chemical Name
aldrin	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,-4-endo-exo-5,8-dimethanonapthalene
chlordane	1,2,3,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7 methanoindene
naled (Dibrom(R))	1,2-dibromo-2,2,dichloroethyl dimethyl phosphate
dieldrin	Not less than 85% of 1,2,3,4,10,10-hexachloro 6,7 epoxy-1,4,4a,5,6,7,8,8a octahydro-1,4-endo-exo-5,8 dimethanonapthalene
diuron (Karmex(R))	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
(Dursban(R))	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene
azinphosmethyl (Guthion(R))	O,O-dimethyl S (4-oxo-1,2,3 benzotriazin-3 (4H) yl-methyl) phosphorodithioate
heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindene
lindane	99+% gamma isomer of 1,2,3,4,5,6-hexachloro-hexane
malathion	O,O-dimethyl phosphorodithioate of dimethyl mercaptosuccinate
methoxychlor	1,1,1-trichloro-2,2,bis (p-methoxyphenyl) ethane
endosulfan Thiodan(R)	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
toxaphene	Chlorinated camphene containing 67-69% chlorine
trifluralin	a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine

in Table 2 (rainbow trout) and Table 3 (bluegill). During the first 24 hours of exposure the susceptibility of fish to most compounds tested increased as temperature increased. Exceptions were the susceptibility of bluegills to lindane and azinphosmethyl, which was unaffected by temperature over the range tested and the susceptibility of both species to methoxychlor which decreased as temperature increased. The effect of temperature on the susceptibility of both species to the test compounds was generally less after 96 hours of exposure than after 24 hours of exposure, the notable exception was the susceptibility of rainbow trout to Trifluralin.

Discussion

There was generally an increase in the susceptibility of fish to most pesticides tested as temperature increased. One might speculate that a very probable mechanism involved here is a higher rate of pesticide uptake at the higher temperatures than at the lower temperature. Because of the limited amount of total available pesticide in the test container, an effect of temperature mediated through pesticide uptake would be expected to be less after 96 hours than after 24 hours; such a trend was evident for most compounds. In certain cases the increased susceptibility of fish to a pesti-

TABLE 2

TL_{50} values (micrograms active ingredient/liter) for rainbow trout (*Salmo gairdneri* Richardson) tested against indicated pesticides, and ranked by the relative increase in susceptibility (R.I.S.) of the fish to the pesticides over the range of temperatures tested.

Compound	Temperature, °C 1.6	7.2	12.7	R.I.S.
		24 Hours		
Dursban(R)	550(480-630)*	110(90-130)	53(45-62)	10.37
Dibrom(R)	1300(1100-1500)	620(570-680)	240(210-270)	5.41
Endrin	15(12-18)	5.3(4.6-6.0)	2.8(2.5-3.1)	5.35
Dieldrin	13(9-19)	3.1(2.6-3.6)	3.1(2.7-3.5)	4.19
Thiodan(R)	13(11-15)	6.1(5.6-6.6)	3.2(2.9-3.5)	4.15
Aldrin	24(18-31)	8.1(6.7-9.7)	6.8(6.0-77)	3.52
Trifluralin	3.8(270-375)	239(196-267)	98(85-113)	3.24
Guthion(R)	25(22-28)	15(12-18)	13(10-15)	1.92
Heptachlor	17(14-21)	12(10-14)	13(12-14)	1.31
Methoxychlor	55(50-60)	45(41-49)	74(66-82)	0.82
		96 Hours		
Dursban(R)	51(43-60)	15(13-17)	7.1(6.0-8.4)	7.18
Trifluralin	210(182-240)	152(132-175)	42(38-46)	5.00
Endrin	2.5(2.2-2.8)	1.4(1.2-1.6)	1.1(0.9-1.2)	2.27
Dibrom(R)	340(310-370)	220(200-230)	160(150-170)	2.12
Thiodan(R)	2.6(2.3-2.9)	1.7(1.5-1.9)	1.5(1.3-1.7)	2.00
Dieldrin	2.4(2.1-2.7)	1.1(1.0-1.2)	1.4(1.3-1.5)	1.71
Aldrin	3.2(2.3-4.5)	3.3(2.8-3.9)	2.2(2.1-2.3)	1.45
Guthion(R)	6.8(6.1-7.5)	6.2(5.3-7.2)	5.5(4.9-6.1)	1.23
Heptachlor	7.7(6.5-9.1)	7.0(6.4-7.6)	7.3(6.9-7.6)	1.05
Methoxychlor	30(28-3.2)	42(38-45)	62(57-67)	0.48

* 95% confidence interval in parentheses

TABLE 3

TL_{50} values (micrograms active ingredient/liter) for bluegills (*Lepomis macrochirus* Rafinesque) tested against the indicated pesticides, and ranked by the relative increase in susceptibility (R.I.S.) of the fish to the pesticides over the range of temperatures tested.

	Temperature, °C			
Compound	12.7	18.3	23.8	R.I.S.
		24 Hours		
Trifluralin	540(460-640)*	360(300-430)	130(110-150)	4.15
Aldrin	36(31-42)	16(14-18)	10(7.8-13)	3.60
Endrin	2.8(2.6-3.0)	1.5(1.3-1.8)	0.8(0.7-1.0)	3.50
Diuron	27,000(25,000-29,000)	17,000(16,000-19,000)	9,700(9,100-10,000)	2.78
Dieldrin	39(34-43)	24(21-27)	15(14-17)	2.60
Chlordane	220(200-240)	170(160-180)	95(83-110)	2.31
Malathion	220(200-240)	140(120-160)	110(97-120)	2.00
Toxaphene	9.7(8.4-11)	6.8(6.2-7.5)	6.6(5.5-7.9)	1.46
Lindane	100(84-120)	100(90-110)	95(86-110)	1.05
Guthion(R)	16(14-18)	16(14-18)	16(15-18)	1.00
Methoxychlor	58(52-64)	67(60-74)	83(77-90)	0.69
		96 Hours		
Trifluralin	190(160-230)	120(100-140)	47(40-55)	4.04
Malathion	120(67-210)	55(51-59)	46(40-52)	2.60
Dieldrin	17(15-19)	14(12-16)	8.8(8.1-9.5)	1.93
Aldrin	7.7(6.8-8.7)	5.8(4.9-6.8)	4.6(4.1-5.1)	1.67
Endrin	0.61(0.54-0.68)	0.41(0.36-0.46)	0.37(0.34-0.40)	1.64
Guthion(R)	6.9(6.2-7.7)	7.4(6.4-8.6)	4.2(3.8-4.6)	1.64
Diuron	8900(8200-9600)	7600(7000-8200)	5900(5300-6500)	1.50
Lindane	54(51-57)	51(46-57)	37(34-40)	1.45
Toxaphene	3.2(2.8-3.7)	2.6(2.2-3.0)	2.4(2.1-2.7)	1.71
Chlordane	85(77-94)	77(69-86)	77(69-86)	1.10
Methoxychlor	42(40-44)	53(51-55)	75(71-79)	0.56

* 95% confidence interval in parentheses

cide could be related to an increased level of enzymatic activity at higher temperatures than at a lower temperature. Examples of this could be the epoxidation of aldrin to dieldrin in bluegills, and the activation of the phosphorothioate Dursban to its phosphate analog in rainbow trout, although Smith et al., (5) have shown that the latter does not occur in goldfish. There also may be an indirect effect of temperature on the susceptibility of fish to pesticides mediated by the effect of temperature on metabolism. As temperature increases so does metabolism. Under the test conditions, this would result in lower dissolved oxygen levels and higher concentrations of waste products at the higher temperatures than at the lower temperature conditions which probably tend to increase the susceptibility of fish to pesticides. However, an effect of temperature mediated in such a manner would be expected to be greater after 96 hours than after 24 hours, and, as we previously noted, the effect of temperature on the susceptibility of fish to pesticides generally decreases with time.

The observed decrease in the susceptibility of fish to methoxychlor as temperature increases seems unusual and is apparently related to chemical structure. Metcalf (6) refers to the increased mortalities at lower temperatures for insects treated with methoxychlor and chemically similar DDT as a negative temperature coefficient of activity. Cope (7) reported a similar phenomenon with rainbow trout exposed to DDT for 24 hours.

Although the processes resulting in increased susceptibility of fish to pesticides at higher temperatures are not completely understood, the general effect of temperature is evident and emphasizes the need for considering interaction between pesticides and environmental factors when determining safe levels of such compounds in aquatic habitats.

Acknowledgments

The authors wish to express their thanks to Austin K. Andrews, William H. Babcock, Walter R. Bridges, Terrence L. Cockrell, Bruce Dart, Edward L. David, John H. Gerdes, John R. McKnight, Jay Stock, and Russell Earnest for their assistance in conducting the toxicity tests.

Literature Cited

1. W. R. BRIDGES, U. S. Public Health Service, PHS-999-WP-25 Trans. 3rd Seminar on Biol. Problems in Water Pollution (1965).

2. M. A. MAHDI, U. S. Bur. Sport Fish. Wild. Resource Publ. #10 (1966).

3. C. R. WALKER, Weeds, 11,226 (1963).

4. J. T. LITCHFIELD and F. WILCOXON, J. Pharmacol. Exptl. Therap. 96, 99 (1949).

5. G. N. SMITH, B. S. WATSON, and F. S. FISCHER, J. Econ. Ent. 59, 1464 (1966).

6. R. L. METCALF, Organic Insecticides, Interscience Publishers Inc. New York, (1955).

7. O. B. COPE, U. S. Bur. Sport Fish. Wild. Serv. Cir. 226 (1965).