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Oxygen Consumption in *Lepomis machrochirus* Exposed to 2,4-D or 2,4,5-T

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Herbicides are released into waterways through direct application for aquatic weed control and through runoff (BOVEY et al. 1974). Concentrations of herbicides in water are usually a few ppb although several ppm may be present shortly after application (AVERITT and GANGSTAD 1976, WHITE et al. 1976, WOJTALIK et al. 1971).

Several investigators have examined the effects of herbicides on fish. Observations of fish made in the days following herbicide application indicate little effect or uptake of herbicide (WOJTALIK et al. 1971). Ester formulations are usually more toxic than salts (MULLISON 1970). Young fish are less tolerant to herbicides than older fish (FRANK 1972). A 24-h LC_{50} of 14.5 ppm 2,4-D has been reported for bluegill (CROSLEY and TUCKER 1966). REHWOLDT et al. (1977) reported TL_m (24-h) values ranging from 55.5 ppm to 306 ppm for a variety of fishes exposed to 2,4-D. They obtained values ranging from 27.3 ppm to 69.3 ppm for these fishes exposed to 2,4,5-T. Chronic exposure to 0.1 ppm of 2,4,5-T resulted in no observable symptoms. JONES (1975) observed no effect on fish exposed to 10 ppm of 2,4-D for three days.

The purpose of this study was to examine metabolic response soon after exposure to 2,4-D and 2,4,5-T and to determine short-term uptake by Lepomis macrochirus (bluegill sunfish). Short time periods were used because herbicides dissipate readily and fish would only be exposed to relatively high concentrations soon after application.

METHODS

Lepomis ranging in wet weight from 0.28-23.30 g were collected from two local ponds using a ¼ inch mesh seine. The fish were randomly assigned to treatments. Fish were transferred in insulated boxes and maintained

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in the laboratory in aquaria. The water used was a mixture of pond water and tap water which had been conditioned by aerating for several days. Fish were transferred from aquaria to 4 L containers for acclimation overnight to 20, 25, or 30°C and 12 light:12 dark photoperiod. Each container housed a single fish of weight greater than 2 g or 4 fish of weight less than 2 g.

Respiration was measured using a closed system and the azide modification of the Winkler technique (APHA 1971). Plexiglass boxes of approximately 200 mL capacity were used for fish less than 2 g wet weight, and 1200 mL capacity for larger fish. The lids were supplied with four screws and were made water tight by a silicone grease seal applied before filling. The boxes were filled with water in which fish were maintained at 20, 25, and 30°C. Solutions of 3 ppm of 2,4-D and 2,4,5-T (butoxyethanol esters)[1] were prepared from this water immediately before respiration was determined. Fish were placed in the respirometry chambers and the chambers were sealed after air bubbles were eliminated. Dissolved oxygen was determined from the stock supply of water used to fill the chambers. All water was transferred by siphoning in order to minimize aeration. The respirometers were placed in incubators for 30-60 min. The largest fish were removed after 30 min and the smallest after one h. Intermediate sizes were removed after 40 min. The time varied in order to prevent excessive oxygen depletion. Respirometers were opened after the appropriate time interval and water drained for oxygen determination. Live wet weights were measured with a triple beam balance for larger fish or a top loading Mettler for very small fish. Respiration is expressed as mL O_2 corrected to STP/h.

Herbicide uptake was determined[2] on fish maintained for 8 days in 4-L containers at 20, 25, or 30°C in 3 ppm of 2,4-D or 2,4,5-T. Fish were sacrificed by quick freezing in order to minimize disturbance of herbicide in their tissue. Whole fish were homogenized in 1N KOH in methanol and refluxed for two h. Samples were filtered in GFA glass fiber filters and washed with methanol. Combined filtrate and washes were evaporated to near dryness. The pH was adjusted to <2 using 10% H_2SO_4. Samples were extracted with ethyl ether/petroleum ether (50/50). The combined organic phases from three extractions were extracted with 2N NaOH. This

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[1]Herbicides were supplied by Amchem Products, Ambler, PA.
[2]Determinations were made by Analytical BioChemistry Lab, Columbia, MO.

basic extract was adjusted to a pH <2 using 10% H_2SO_4 and then extracted using chloroform. The chloroform was then evaporated to near dryness and the residue methylated using diazomethane. A Florisil column cleanup was used. The concentration of 2,4,5-T was determined using a GLC with a 2% OV-210/1.5% OV-17 column (column temperature--205°C, inlet temperature--220°C, ^{63}Ni detector temperature--300°C). The 2,4-D concentration was determined using a 10% silar 10C column (column temperature--180°C, inlet temperature--200°C, ^{63}Ni detector temperature--180°C). Recovery of 77% was determined for 2,4-D and 81% for 2,4,5-T.

RESULTS

A stepwise multiple regression (N=88) was used to test the importance of herbicides, temperature, and fish weight in predicting log-transformed respiration. Weight ($P<0.0001$) and temperature ($P<0.006$) were both significant variables for predicting respiration ($r^2=0.93$). However, 2,4-D, 2,4,5-T, or control ($P<0.22$) treatment did not contribute significantly to the predictive value of the regression. Therefore, data for the herbicides were combined and the relationship between log respiration and log weight was examined using simple regressions for each temperature. The intercept was significantly larger for 30°C data although slope did not vary significantly among the three temperatures (Table 1).

TABLE 1

Intercepts, Slopes, and Predictive Ability of Regressions of Log(Oxygen Consumption) on Log(Weight). Standard errors are given in parentheses.

TEMPERATURE (°C)	INTERCEPT	SLOPE	r^2
20	-1.63 (0.12)	0.97 (0.06)	0.88
25	-1.63 (0.14)	1.05 (0.08)	0.88
30	-0.92 (0.11)	0.73 (0.07)	0.80

Herbicide uptake was quite small (Table 2). Control fish taken from the stock supply of fish all showed <0.05 ppm of herbicide (N=5).

TABLE 2

Mean Uptake of Herbicides by Whole Fish Maintained in 3 ppm of 2,4-D or 2,4,5-T for Eight Days Prior to Sacrifice. The number of determinations is given in parentheses.

TEMPERATURE (°C)	2,4-D MEAN UPTAKE	2,4,5-T MEAN UPTAKE
20	<0.05 (5)	0.08 (3)
25	<0.05 (4)	0.12 (4)
30	<0.05 (1)	0.06 (2)

DISCUSSION

The log transformation allows comparison with other fish respiration data. Log-transformed respiration versus log-transformed weight usually gives a line with a mean slope of 0.85 (FRY 1957). This reveals that the relationship between respiration and weight is intermediate between surface area and weight dependence (FRY 1957). FRY gave slopes between 0.78 and 0.90 for different studies. The slopes of the regression equations with log-transformed data for this study were approximately in that range (0.73-1.05). Slopes did not differ significantly among the three temperature groups. These data are consistent with the hypothesis that the response of respiration to weight is independent of temperature (FRY 1957). The influence of temperature on respiration is evident from the intercepts with elevated respiration at 30°C.

More 2,4,5-T than 2,4-D was retained by the fish. Since 2,4-D is more rapidly degraded by microorganisms, less would be available for uptake (ALEXANDER and ALLEN 1961). The smaller uptake of 2,4,5-T at 30°C may result from reduced availability of the herbicide due to accelerated degradation at higher temperatures (AVERITT and GANGSTAD 1976). Phenoxy herbicides are generally not retained as effectively as less polar pesticides (ISENSEE 1976).

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REFERENCES

ALEXANDER, M. and M. I. H. ALLEN: J. Agri. Food Chem. 9, 44(1961).

APHA: Standard Methods. Amer. Public Health Assoc. 1971.

AVERITT, W. L. and E. O. GANGSTAD: J. Environ. Qual. 5, 145(1976).

BOVEY, R. W., E. BURNETT, C. RICHARDSON, M. G. MERKLE, J. R. BAUER, and W. G. KNISEL: J. Environ. Qual. 3, 61(1974).

CROSLEY, D. G. and R. K. TUCKER: Science 154, 289(1966).

FRANK, P. A: Herbicide Residues in Aquatic Environments. Amer. Chem. Soc. 1972.

ISENSEE, A. R.: Intern. J. Environ. Studies 10, 35 (1976).

JONES, W. E.: Water Treatment and Contam. 24, 132 (1975).

MULLISON, W. R.: Weed Science 18, 738(1970).

REHWOLDT, R. E., E. KELLY, and M. MAHONEY: Bull. Environ. Contam. Toxicol. 18, 361(1977).

FRY, F. E. J.: Aquatic Respiration of Fish. In: The Physiology of Fishes, Vol. 1. New York: Academic Press, 1957.