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A Preliminary Study of the Effects of Diflubenzuron and Methoprene on Rainbow Trout (*Salmo gairdneri* Richardson)

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INTRODUCTION

Recent research has shown insect growth regulators to be environmentally less objectionable than many older insecticides (MIURA and TAKAHASHI 1975, 1974; NORLAND and MULLA 1975). Diflubenzuron (Dimilin; 1-(4-chlorophenyl)-3(2,6-diflurobenzoyl) urea; Thompson-Hayward Company) and Methoprene (Altosid; isopropyl-11-methoxy-3, 7, 11-trimethyldodeca-2, 4-dienoate; Zoecon Corporation) are two insect growth regulators which are expected to be used on a large scale within the next few years. During the course of their use in insect control programs, non-target organisms, including fish will be exposed to these compounds.

Exposure of fish to Methoprene has produced LC_{50} values ranging from 3.3 mg/l for trout (species not specified) to >100 mg/l for channel catfish (*Ictalurus punctatus* (Rafinesque)) (ANON. 1973). For Diflubenzuron, LC_{50} values range from 135 mg/l for bluegill sunfish (*Lepomis macrochirus* Rafinesque) to 255 mg/l for the salt water minnow (*Fundulus heteroclitus* Linnaeus) (ANON. 1975). These values are well above concentrations expected as a result of normal insect control operations ie. ≤0.05 mg/l (ANON. 1975; ELINGS and DIEPERINK 1974; SCHAEFFER and WILDER 1973, 1972; SCHAEFER, *et al.* 1975). Therefore, acute fish toxicity would not be expected during control programs, although sublethal effects could occur. In the absence of published literature describing sublethal effects, or even suggesting their existence, several blood serum parameters commonly used to study various stress responses in fish (LEIVESTAD and MUNIZ 1976; LARSSON and LEWANDER 1973; WARDLE 1972; EISLER 1967, BELL 1968; MENTON 1927) were measured subsequent to treatment of rainbow trout (*Salmo gairdneri* Richardson) with Diflubenzuron or Methoprene.

MATERIALS AND METHODS

Rainbow trout (9.7 ± 3.3 g; raised at the Freshwater Institute's Rockwood Hatchery) were acclimated at 15°C and a 12:12 photoperiod in dechlorinated Winnipeg city tap water (hardness as $CaCO_3$, 90 mg/l; conductivity 190 µS/cm; pH 7.9) for 3 weeks prior to treatment. Twenty-four hours before exposure the once daily feeding schedule was stopped and 10 fish of either sex were randomly distributed into each of 11, 20-liter glass aquaria. A continuous flow diluter (HARRISON, *et al.* 1975) with a 95% replacement time of 2.3 hours was used to deliver Diflubenzuron as Dimilin WP-25, or Methoprene as Altosid SR-10 at theoretical concentrations of 0.625,

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1.25, 2.5, 5.0, and 10 mg/l to the aquaria. (If no effects were noted at these high concentrations we assumed that effects would not be revealed by the same analyses at normal application rates.) Two aquaria were treated at each concentration with one aquarium left as a control receiving only dechlorinated water. During treatment temperature of the aquaria was 15 $\pm$ 0.5°C and dissolved oxygen was maintained near saturation by vigorous aeration.

After 96 hours, 5 fish per aquarium (10 from the control) were weighed and bled from caudal vessels into capillary tubes. Hematocrit was determined after centrifugation for 3 minutes in a Clay Adams Autocrit centrifuge. Plasma was removed by breaking the hematocrit capillary; after clotting, the serum was analyzed for sodium, lipid, glucose and glutamate oxalacetate transaminase using methods described in MATTENHEIMER (1970) and reagents from Boehringer Mannheim Corp. (BMC Diagnostics Ltd., Montreal). Only the sera from fish in the 10 mg/l and control aquaria were collected during the Methoprene trial, and an effect upon blood glucose was detected; consequently a second Methoprene trial was performed to determine whether the glucose effect was dose dependent.

All serum data were logarithmically transformed in order to decorrelate means and variances prior to statistical analyses. Statistical differences in means were determined by the "Student" t-distribution.

RESULTS AND DISCUSSION

Blood measurements for the fish are shown in Tables 1-3. It is noteworthy that apparently similar groups of fish were biochemically distinct as indicated by the differences between control groups However, comparisons within Diflubenzuron or Methoprene groups show little variation.

The results from serum analyses of fish exposed to Diflubenzuron are shown in Table 1. A statistical difference ($P \leq 0.025$) was found between the 10 mg/l and control glutamate oxalacetate transaminase (GOT) serum values. As a result, sera from fish at each concentration were analyzed for this enzyme (Table 2) and a statistically significant dose related effect was evident. Regression analyses of $\log_{10}$ transformed GOT values on $\log_{10}$ transformed Diflubenzuron concentrations (control concentration 0.01 mg/l) produced the linear relationship defined by the equation $Y = -0.079X + 2.511$ with $P \leq 0.01$ and $r^2 = .22$ ($n-1 = 59$). Despite the drop in GOT values of about 50%, serum activities for GOT were within the range previously reported for this enzyme in rainbow trout from the same hatchery ie. 65-803 mU/ml (LOCKHART *et al.* 1973) and we are uncertain of the pathological significance, if any, of this change.

During exposure to Methoprene the rainbow trout in the treated aquaria became visibly lethargic in comparison with the control fish. Analyses of sera from fish exposed to Methoprene are shown in Table 1. A signfiicant difference ($P \leq 0.01$) was found between the 10 mg/l and control glucose serum values and in the second Methoprene trial a decrease in blood glucose values was

TABLE 1

Effect of Diflubenzuron and Methoprene on rainbow trout blood serum chemistry. Values are means with 95% confidence intervals determined from log transformed data

	Diflubenzuron		Methoprene	
	Treated (10 mg/l)	Control	Treated (10 mg/l)	Control
Haematocrit (%)	25.9(23.1, 28.8)	24.1(21.7, 26.6)	35.1(31.6, 38.8)	32.9(30.1, 35.9)
Lipid (mg/dl)	359.2(158.9, 811.5)	596.4(427.4, 831.9)	824.9(272.2, 2499.0)	997.6(136.0, 1609.7)
GOT[1] (mU/ml)	213.8*(112.6, 405.8)	430.7(359.0, 516.6)	428.9(306.6, 599.8)	423.8(311.5, 576.7)
Glucose (mg/dl)	71.9(51.9, 99.7)	67.2(63.3, 68.8	37.2**(31.5, 44.0)	64.8(61.0, 68.9)
Sodium (mg/dl)			350.5(333.6, 368.3)	357.3(342.5, 372.7)

[1]Glutamate oxalacetate transaminase (aspartate aminotransferase; E.C. 2.6.1.1).
* Statistically different from control at $p \leq .025$.
**Statistically different from control at $P \leq .01$.

TABLE 2

Effect of five concentrations of Diflubenzuron on rainbow trout blood serum GOT[1] activities. Values are means with 95% confidence intervals determined from log transformed data

Diflubenzuron mg/l	$\bar{x}$ (mU/ml)
10	213.8 (112.6, 405.8)
5	293.5 (218.9, 393.4)
2.5	298.2 (241.6, 368.1)
1.25	367.8 (291.5, 464.1)
0.625	342.1 (235.8, 496.3)
Control	430.7 (359.1, 516.6)

[1]Glutamate oxalacetate transaminase (Aspartate aminotransaminase; E.C. 2.6.1.1).

noted in all treated fish (Table 3). However, this decrease did not significantly change at concentrations above 1.25 mg/l. Therefore, despite treatments of 2.5, 5.0 and 10 mg/l, the actual

TABLE 3

Effect of five concentrations of Methoprene on rainbow trout blood serum glucose values

Methoprene (mg/l)	$\bar{x}$ (mg/dl)
10	41.8 (34.9, 50.0)[1]
5	38.1 (33.6, 43.2)
2.5	36.6 (28.4, 47.1)
1.25	42.2 (36.5, 48.9)
0.625	50.9 (50.2, 58.1)
Control	60.0 (53.1, 67.9)

[1]95% confidence intervals as determined from log transformed data.

concentration of Methoprene in these aquaria was probably 1.39 mg/l, ie. the maximum solubility of Methoprene in water (ANON. 1973). Regression analyses of $\log_{10}$ transformed glucose values on $\log_{10}$ transformed Methoprene concentrations (assuming the 2.5, 5.0 and 10 mg/l aquaria had an actual concentration of 1.39 mg/l; the control concentration 0.01 mg/l) resulted in the linear equation $Y = -0.080X + 1.629$ with $P \leq 0.01$ and $r^2 = .31$ $(n-1 = 59)$. This indicates a dose dependent decrease of rainbow trout blood serum glucose concentrations which continued until the maximum solubility of Methoprene was reached. While these concentrations of Methoprene are relatively high, the value of blood sugar measurements as indicators of "stress" in fish has been emphasized recently by Silbergeld (1974).

These empirical observations are being reported now partly in the hope that they may suggest future experiments in toxicology and partly since they offer some potential as a monitoring tools to detect sublethal exposures of fish following operational use of these materials.

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