Toxicity of Paired Mixtures of Candidate Forest Insecticides to Rainbow Trout

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Pesticide mixtures that have greater than additive toxicity (synergism) may produce unexpected hazards to fish and wildlife. Examples of greater than additive toxicity to mammals were reported by FRAWLEY (1965) and to insects by EL SABAE *et al.* (1964) and LICHTENSTEIN *et al.* (1973). KREITZER and SPANN (1973) reported that pheasants and quail are less susceptible than mammals or insects to synergism of some pesticides and polychlorinated biphenyls. In their studies, malathion was a component of the most synergistic mixtures.

Fish are frequently exposed to more than one pesticide because some persist in the environment, some are applied repeatedly, and other are applied as combinations to increase efficacy or reduce cost (HOWELL *et al.* 1964; BROWN and NISHIOKA 1967; and MOUBRY *et al.* 1968). Chemical combinations altered the tissue storage capacity for DDT in exposed rainbow trout (MAYER *et al.* 1970), as well as the toxicity to fish (HERBERT and SHURBEN 1964; LLOYD 1961). FERGUSON and BINGHAM (1966) reported that the toxicities of certain mixtures of pesticides to fishes were additive or less than additive, and that results were influenced by the use of susceptible or resistant populations of fish. Therefore, a need exists to identify groups of chemicals or individual pesticides that may cause unexpected toxic effects when they are used in the presence of other pesticides.

Seven candidate forest insecticides were chosen for testing in paired mixtures against rainbow trout *(Salmo gairdneri)*. The compounds include a carbamate, organophosphates, and pyrethroids. Except for two experimental synthetic pyrethroids, all are registered insecticides. This study was designed to determine the individual and additive toxicities of these insecticides to rainbow trout, to quantify the additive toxicity by an index, and to define the significance of additive toxicity in terms of ranges for the index.

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Materials and Methods

Rainbow trout were obtained from a Federal fish hatchery, maintained by a fish culturist prior to use, acclimated to test waters, and exposed to chemicals in standardized soft water at 12° C as outlined by HUNN *et al.* (1968) and MARKING (1969). The trout were exposed to the individual insecticides and to the mixtures on the same day and under the same test conditions in an effort to minimize the variability of response.

Chemicals tested included Zectran(R) (a carbamate); Dylox(R), Volaton(R), and Guthion(R) (organophosphates); and pyrethrum extract, SBP-1382(R), and RU-11679 (pyrethroids). All chemicals were technical grade; however, technical grade pyrethrum extract contains 20% pyrethrins and 80% inert ingredients. Stock solutions were prepared by dissolving the compounds in acetone, and portions of each stock were added to static test solutions to yield selected concentrations of each compound. Dead fish were recorded and removed daily throughout the 96-hour tests.

Toxicities of individual insecticides were determined according to standardized laboratory procedures described by LENNON and WALKER (1964). The toxicities of paired mixtures were determined similarly to individual chemicals except that the two chemicals were added in a one to one ratio of their LC50's. LC50's were calculated according to the method of LITCHFIELD and WILCOXON (1949). Toxicity of each mixture of chemicals was evaluated by summing the toxic contributions in the mixture and converting the sum to a linear index value (DAWSON and MARKING 1973). A summary of the procedure follows:

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S$$ (the sum of biological effect), where A and B

represent chemicals; i and m are the individual and mixture toxicities (LC50's), respectively.

$$\text{Additive index} = \frac{1}{S} - 1.0 \text{ for } S \leq 1.0 \text{ and}$$

$$\text{Additive index} = [S(-1)] + 1 \text{ for } S \geq 1.0$$

A range for each additive index was derived by substituting values of the 95% confidence intervals for LC50's (MARKING and DAWSON[1]). Ranges that overlap zero indicate additive toxicity. Positive ranges indicate greater than additive toxicity, and negative ranges indicate less than additive toxicity.

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[1] A method to Assess the Toxic or Other Effects of Mixtures of Chemicals. Manuscript in review. Fish Control Laboratory, La Crosse, Wisconsin.

Results and Discussion

Zectran, a carbamate insecticide, was the least toxic of the chemicals tested; the 96-hour LC50 was 20.0 mg/ℓ (Table 1). Organophosphates Dylox and Volaton ranked next in order of toxicity, followed by pyrethrum extract and Guthion. The synthetic pyrethroids, SBP-1382 and RU-11679, were most toxic of the group with 96-hour LC50's of 2.20 and 0.550 μg/ℓ, respectively.

Paired mixtures of the seven insecticides produced toxicity to rainbow trout that ranged from less than additive to greater than additive. Additive indices ranged from -0.945 for the mixture of Zectran and Volaton to +0.672 for Volaton and RU-11679 (Table 2). Nine of the mixtures had additive index ranges that were less than zero; their toxicity was less than additive (antagonistic). Nine others had additive index ranges that overlapped zero; their toxicity was simply additive. Two mixtures had additive index ranges that were greater than zero; their toxicity was greater than additive (synergistic). Neither the mixtures producing greater than additive toxicity nor those producing less than additive toxicity showed marked deviations from additive toxicity.

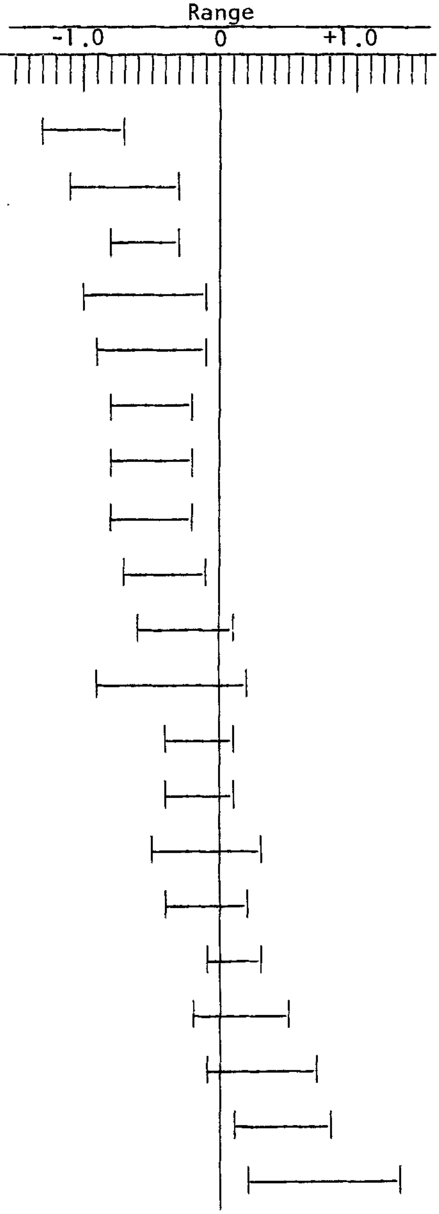
TABLE 1

Toxicity of Insecticides to Rainbow Trout in Soft Water at 12° C

Compound	Unit	96-hour LC50 and 95% confidence interval
Zectran	mg/ℓ	20.0 18.7-21.4
Dylox	mg/ℓ	4.85 4.31-5.46
Volaton	μg/ℓ	349 319-381
Pyrethrum	μg/ℓ	52.2 48.2-56.5
Guthion	μg/ℓ	7.10 6.28-8.03
SBP-1382	μg/ℓ	2.20 1.81-2.68
RU-11679	μg/ℓ	0.550 0.4783-0.640

Additive Toxicity of Paired Insecticides to Rainbow Trout

Mixture	Additive index
Zectran, Volaton	-0.945
Dylox, RU-11679	-0.659
Dylox, Pyrethrum	-0.523
Volaton, SBP-1382	-0.518
Dylox, SBP-1382	-0.466
Zectran, Pyrethrum	-0.459
Zectran, Dylox	-0.448
Zectran, Guthion	-0.443
Guthion, Pyrethrum	-0.390
Guthion, SBP-1382	-0.245
Zectran, SBP-1382	-0.231
Guthion, RU-11679	-0.137
Volaton, Pyrethrum	-0.121
Zectran, RU-11679	-0.085
Dylox, Volaton	-0.059
RU-11679, Pyrethrum	+0.085
SBP-1382, Pyrethrum	+0.145
SBP-1382, RU-11679	+0.278
Dylox, Guthion	+0.419
Volaton, RU-11679	+0.672



The synergism of pyrethroids against insects and mammals has been attributed mainly to interference with enzyme systems (O'BRIEN 1967; CASIDA 1973). The synergizers generally were solvents or drugs rather than other insecticides; however, any combination of chemicals that stimulates or inhibits enzyme systems may produce unexpected biological effects. Organophosphates have been synergized by other organophosphates (DUBOIS 1961). Additive toxic effects of chemicals to fish are unpredictable and complex. The possible toxic combinations of various chemicals that either may appear or be applied sequentially or simultaneously in the environment should be further investigated to identify hazardous mixtures of chemicals.

Summary

The toxicities of Zectran, Dylox, Volaton, Guthion, pyrethrum extract, and the synthetic pyrethroids SBP-1382, and RU-11679 were determined individually and in paired mixtures against rainbow trout. The carbamate Zectran was the least toxic, and the synthetic pyrethroids were the most toxic. Nine of the mixtures produced less than additive toxicity (antagonism), nine produced additive toxicity, and two produced greater than additive toxicity (synergism). None of the mixture toxicities deviated markedly from additive, and only two pairs of mixtures would pose a greater toxicological hazard to fish than the respective individual chemicals.

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