Nutritional Effects on Chlordane Toxicity in Rainbow Trout

by
PAUL M. MEHRLE,
W. WAYNON JOHNSON, and
FOSTER L. MAYER, JR.
Fish-Pesticide Research Laboratory
Bureau of Sport Fisheries & Wildlife
U.S. Department of the Interior
Route 1
Columbia, Mo. 65201

Procedures for toxicity tests with fish have been outlined by the AMERICAN PUBLIC HEALTH ASSOCIATION (1971) and LENNON and WALKER (1964). These procedures describe requirements for water quality (pH and hardness), temperature, species, size of fish, handling, and other details necessary for evaluating the toxicity of a chemical to fish. However, an important variable not considered in standard procedures is the type of diet fed to the fish prior to testing. Diet composition alters the oral toxicity of organochlorine, organophosphate, and carbamate pesticides in rats (BOYD 1969; KRIJNEN and BOYD 1971). The studies demonstrated that the feeding of protein deficient diets resulted in a decreased tolerance to the pesticide. Similar results were reported by McCLEAN and McCLEAN (1966) who suggested that dietary protein deficiency decreased liver microsomal detoxifying mechanisms. In contrast, MIRANDA and WEBB (1972) reported that the oral toxicity of heptachlor was less in rats fed the low quality protein, gluten, than in rats fed casein. Thus, both qualitative and quantitative alterations in dietary proteins can affect the acute toxicities of pesticides in rats.

Investigations of similar nutritional-toxicological interactions in fish have not been reported. The present study was designed to determine the effect of different diets, both commercial and synthetic, on the tolerance of rainbow trout (Salmo gairdneri) to chlordane as determined by acute toxicity tests.

MATERIALS AND METHODS

Rainbow trout weighing 0.4 ± 0.1 g were maintained for 42 days in groups of 300 in each of six 80-liter glass aquaria with an inflow of one l/min. Water temperature was 17 ± 0.5°C. A constant photoperiod of 16 hours per day was maintained with overhead fluorescent lighting. The fish were maintained in aerated well water with the following characteristics: pH 7.4; alkalinity, 237 mg/l; total hardness, 272 mg/l.

Each group of 300 fish was fed one of four commercial diets (Table 1) or one of two synthetic diets (Table 2) at the rate of 3% of their body weight daily for 42 days. Ration adjustments for growth were made weekly. Mortalities were recorded and removed daily. At the end of the feeding period, static toxicity tests were conducted according to the methods outlined by LENNON and

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WALKER (1964). The tests were conducted at 17 ± 0.5°C in five-gallon glass jars containing 15 liters of reconstituted water. The reconstituted water (MARKING 1969) had the following chemical characteristics: pH, 7.4; alkalinity, 35 mg/l; total hardness, 40 mg/l. Ten fish were exposed to each of ten concentrations of chlordane for each diet group. Aliquots of technical grade chlordane dissolved in acetone were pipetted into the reconstituted water to give the desired concentrations. The acetone concentration in test and control solutions was one ml/l. LITCHFIELD and WILCOXON'S (1949) method for analyzing dose-effect experiments was used to determine the 96-hr LC50 values and confidence limits.

TABLE 1

Composition of commercial diets fed to rainbow trout prior to toxicity testing. 1/2/

	Percentage
Ewos High Fat Salmon Starter	
Crude Protein	58.0
Fat	16.0
Ash	11.5
Moisture	5.5
Cellulose	1.5
Carbohydrate	7.5
Glencoe Quality Trout Food	
Crude Protein, not less than	45.0
Crude Fat, not less than	12.0
Crude Fiber, not more than	4.5
Silver Cup Fish Food	
Crude Protein, not less than	40.0
Crude Fat, not less than	5.0
Crude Fiber, not more than	7.0
Ash, not more than	15.0
Oregon Moist Fish Pellets	
Crude Protein, not less than	35.0
Crude Fat, not less than	5.0
Crude Fiber, not more than	4.0
Moisture, not more than	35.0

1/ Composition as listed on product label.

2/ Use of trade names does not constitute endorsement by the Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior.

TABLE 2

Composition of synthetic diets fed to rainbow trout prior to toxicity testing.[1/]

Component	Percentage of Dietary Components by Weight	
	Low protein diet (23% protein)	High protein diet (45% protein)
Casein	23.0	45.0
Dextrin	46.7	24.6
Mineral Mix	3.7	3.7
Alpha-cellulose	6.5	6.6
Alpha-tocopherol	0.2	0.2
Choline chloride	0.9	0.9
Vitamin Mix	1.9	1.9
Salmon Oil	2.6	2.6
Corn Oil	6.5	6.5
Water	8.0	8.0

[1/] Purified diets modified from Castell, et al. (1972).

TABLE 3

Toxicity of chlordane to rainbow trout on various diets.

Diet	96-hr LC50 (µg/l)	95% Confidence interval
Oregon Moist	8.2	6.1 - 11
Glencoe	9.1	4.8 - 17
Silver Cup	20	14 - 28
Ewos	31	23 - 43
Low Protein (23%)	29	20 - 41
High Protein (45%)	47	38 - 59

RESULTS AND DISCUSSION

The LC50 values of chlordane were affected by the type of diet fed prior to exposure (Table 3). A significant (P=0.05) difference is evident between the LC50 values for the groups fed the synthetic diet containing 23% protein and the synthetic diet containing 45% protein. These results concur with those reported by KRIJNEN and BOYD (1971), i.e., low dietary protein increases susceptibility to organochlorine compounds. Fish fed the 45% protein diet grew faster than those fed the 23% protein diet and the commercial diets. Average weight of the fish fed the 45% protein diet was 1.4 g, while the weight of fish in all other groups averaged 1.1 g.

The group receiving the 23% protein diet had a significantly (P=0.05) greater LC50 value than the groups receiving the Glencoe and Oregon Moist diets. There was no discernible difference in LC50 values of fish fed the 23% protein diet, Silver Cup, or the Ewos diets. These results suggest that the quality of protein may be an important factor in increased resistance since the LC50 values were not directly related to protein content of diets. The primary sources of protein in the Glencoe, Silver Cup and Oregon Moist diets were fish meal, soybean meal, cottonseed meal, and wheat germ meal. The ratios of the components, however, were unknown. The main protein source in the Ewos diet was lipid-extracted fish meal (fish protein concentrate). Overall, the 45% protein diet containing casein as the single protein source appeared to have the greatest effect in regard to increasing the resistance of trout to chlordane. The effects of the combination of proteins in the Silver Cup and Ewos diets were similar to that of the 23% protein diet containing casein.

Differences were also observed in the toxicity values among the groups fed commercial diets. A significant (P=0.05) difference existed between the group fed the Ewos diet and the other three commercial diets. There was also a significant (P=0.05) difference between the group fed Silver Cup and the groups fed Glencoe and Oregon Moist diets. The difference in LC50 values, however, between the groups fed Oregon Moist and Glencoe was not significant.

The responses observed among the various groups can be attributed to different inductive potentials of detoxification mechanisms by the various diets. Presumably, the quality and/or quantity of dietary protein is responsible for maintaining homeostatic levels of detoxification enzymes. However, other nutrients such as ascorbic acid contribute to the microsomal detoxification mechanisms in guinea pigs (WAGSTAFF and STREET 1971). Also, the lipid composition (phosphotidylcholine) in liver microsomes of rats is essential for enzymatic activity (STROBEL et al., 1970). The reason for the differences in susceptibility to chlordane among the dietary groups in the present study is not known. This study illustrates, however, that the type of diet fed prior to toxicity testing with fishes is important, and that consideration for a standard diet in toxicological research with fish is needed.

SUMMARY

Rainbow trout were fed the commercial diets Oregon Moist, Glencoe, Silver Cup, or Ewos, and synthetic diets containing 23% or 45% protein. After feeding the diets for 42 days, chlordane was tested against rainbow trout in static toxicity tests. LC50s (96-hr) for chlordane were 8.2, 9.1, 20, 31, 29 and 47 µg/l for the groups fed Oregon Moist, Glencoe, Silver Cup, Ewos diets and synthetic diets that contained 23 and 45% protein, respectively. The LC50 for the group fed the 45% protein diet was statistically (P=0.05) different from the values for all the other groups. Also, the values differed among groups fed commercial diets.

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