Effects of PCB (Aroclor 1254) and p,p'DDT on Production and Survival of *Daphnia Magna* Strauss*

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Introduction

This paper describes the combined and individual effects of p,p'DDT and PCB (Aroclor 1254) on the survival and reproduction of Daphnia magna Strauss in laboratory toxicity tests. The simultaneous occurrence of each of these chlorinated hydrocarbon compounds as residues in biological samples from both freshwater and marine environments has been widely documented (STALLING and MAYER 1972, HOLMES et al. 1967, HOLDEN and MARSDEN 1967, VEITH and LEE 1970). Although the toxicity of each compound has been reported for various forms of aquatic life (HANSEN et al. 1971, WILDISH 1970, ANDERSON 1945) their potential combined or additive toxicity may be of even greater significance.

LICHTENSTEIN et al. (1969) found sublethal levels of several PCB isomers increased the toxicity of DDT and dieldrin to Drosophila melanogaster and adult houseflies Musca domestica. Similar toxicity tests with the housefly demonstrated that the polychlorinated biphenyl preparation Aroclor 1254 is a powerful synergist for the carbamate insecticide carbaryl (PLAPP 1972). Combined toxicity tests with aquatic organisms have not been reported although the simultaneous occurrence of PCB and DDT in aquatic systems is well known (RISEBROUGH et al. 1968).

Methods and Materials

A stock culture of Daphnia magna was maintained in aerated 5 gallon aquaria at 24 ± 1°C under constant illumination. The animals were fed daily with approximately 5 ml of a suspension containing 10.0 gms pulverized trout fry food, and 0.5 gms whole wheat germ blended into 300 ml of filtered well water (BIESINGER and CHRISTENSEN 1972).

Toxicant stock solutions were prepared from an Aroclor 1254 standard (Monsanto Co., St. Louis, Missouri) and a 99+% pure p,p'DDT standard (p,p'1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) in volumetric flasks with reagent grade acetone. Test solutions were made each day by diluting appropriate quantities of the primary stock solutions with water and thoroughly mixing in the test tanks. It was assumed that the addition of the soluble food suspension prior to adding the toxicant aided in the dispersal and solubilization of toxicants in the water.

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The toxicity test chambers were 1000 ml beakers and dilution water was carbon filtered well water with the following general chemical characteristics: pH 7.6, hardness 320-340 ppm as $CaCO_3$, dissolved oxygen 7.8-8.2 ppm. Sufficient dilution water to prepare a single day's test was aerated to saturation prior to preparation of the toxicant solutions.

Prior to a test, adult Daphnia, sorted by size, were isolated in a separate aquarium and the young produced overnight were tested the following day. In this manner known age individuals, 12± 12 hours old were used in all tests. Ten individuals were placed in each test beaker, one ml of the food suspension was added and the beaker was covered with a watchglass to minimize evaporation.

Initial toxicity tests were conducted to determine the toxicity of p,p'DDT and Aroclor 1254 separately prior to tests with combinations of the compounds. Tests were run for 16 days and since production of young usually began after 8 or 9 days this schedule gave at least 7 days of reproductive data for each test. The organisms were pipetted into fresh test solutions every two days until they began producing young after which solutions were renewed daily. The young were also counted, removed and discarded daily. Initial mortality (measured as complete immobilization), average brood size/day, number of days young were produced and total young were recorded. All tests were replicated and a total of 11,620 individual Daphnia and young were tested during these experiments. The results from replicate tests of each concentration were pooled for statistical analysis. The toxicity data are presented in the terminology recommended by SPRAGUE (1970). The 14-day LC_{50} data, 50% effect on reproductive impairment and 95% confidence intervals were calculated according to the methods of LITCHFIELD and WILCOXON (1949).

Results

The preliminary toxicity tests established the two week LC_{50} for DDT and Aroclor 1254 to Daphnia to be 0.67 ppb and 24.0 ppb respectively (Table 1). Of the three reproductive parameters examined, the total young produced over a seven day period proved to have the lowest EC_{50} with 50% inhibition of total young occurring at 0.50 ppb for DDT and 19.0 ppb for PCB. The acute toxicity and average brood size/day, obtained by dividing the number of young by the number of parents, had lower EC_{50}'s than did the percentage of days reproducing (Table 1).

Once the separate effects of DDT and PCB were established, toxicity tests were conducted with combined concentrations of the two toxicants. Test concentrations of Aroclor 1254 were varied between 2 and 24 ppb among several tests while the DDT concentrations were held at 0.30, 0.50, and 0.75 ppb and DDT levels were varied between 0.20 and 0.60 ppb in the presence of a constant concentration of 12 ppb of Aroclor 1254. In all tests conducted, the effects of one toxicant were significantly enhanced by the presence of the other. The presence of a no-effect (12 ppb) level of PCB increased susceptibility of Daphnia to DDT by approximately 1/3 and the presence of 0.5 ppb DDT increased the toxicity of PCB by approximately 2X.

TABLE 1

The concentrations (ppb) of p,p' DDT and Aroclor 1254 alone and in combination that affect survival (LC_{50}) and reproduction (EC_{50}) of Daphnia magna in a 14 day test.

Toxicant Combination	Acute Toxicity 14-day LC_{50}		Reproductive Inhibition EC_{50} (ppb): Total Young		Average Brood Size		Percentage of Days Reproducing	
	mean	C.L.*	mean	C.L.	mean	C.L.	mean	C.L.
p,p' DDT only	0.67	(0.65- 0.69)	0.50	(0.48- 0.52)	0.61	(0.58- 0.64)	0.75	(0.71- 0.79)
DDT with 12 ppb Aroclor 1254	0.40	(0.38- 0.41)	0.30	(0.28- 0.32)	0.45	(0.43- 0.48)	0.55	(0.52- 0.58)
Aroclor 1254 with 0.75 ppb DDT	4.0	(3.80- 4.20)	1.8	(1.69- 1.91)	6.0	(5.82- 6.18)	9.0	(8.65- 9.36)
Aroclor 1254 with 0.50 ppb DDT	12.0	(11.54-12.48)	10.0	(9.17-10.90)	13.0	(11.92-14.17)	11.0	(10.09-11.99)
Aroclor 1254 with 0.30 ppb DDT	14.0	(13.46-14.56)	13.0	(12.26-13.78)	19.0	(18.27-19.76)	19.0	(17.92-20.14)
Aroclor 1254 only	24.0	(23.08-24.96)	19.0	(17.93-20.14)	23.0	(22.12-23.92)	25.0	(23.81-26.25)

*Confidence limits for p = 0.05.

The direction of this additive toxicity is interpreted graphically (Figure 1) according to the terminology suggested by SPRAGUE (1970). The axes of the graphs represent proportions of the 14-day EC_{50} values for acute toxicity, total young, average brood size and percentage of days reproducing. Data points are those combinations of toxicant concentrations that produced the 50% effect level for each test parameter. The diagrams indicate that for all of the concentrations tested, DDT and PCB operate in the less-than-additive interaction area, i.e., the effect of the combined chemicals is somewhat less than would be expected if their effects were exactly additive. The data from a test with a 0.75 ppb concentration of DDT in the presence of low levels of PCB fall outside the inter-

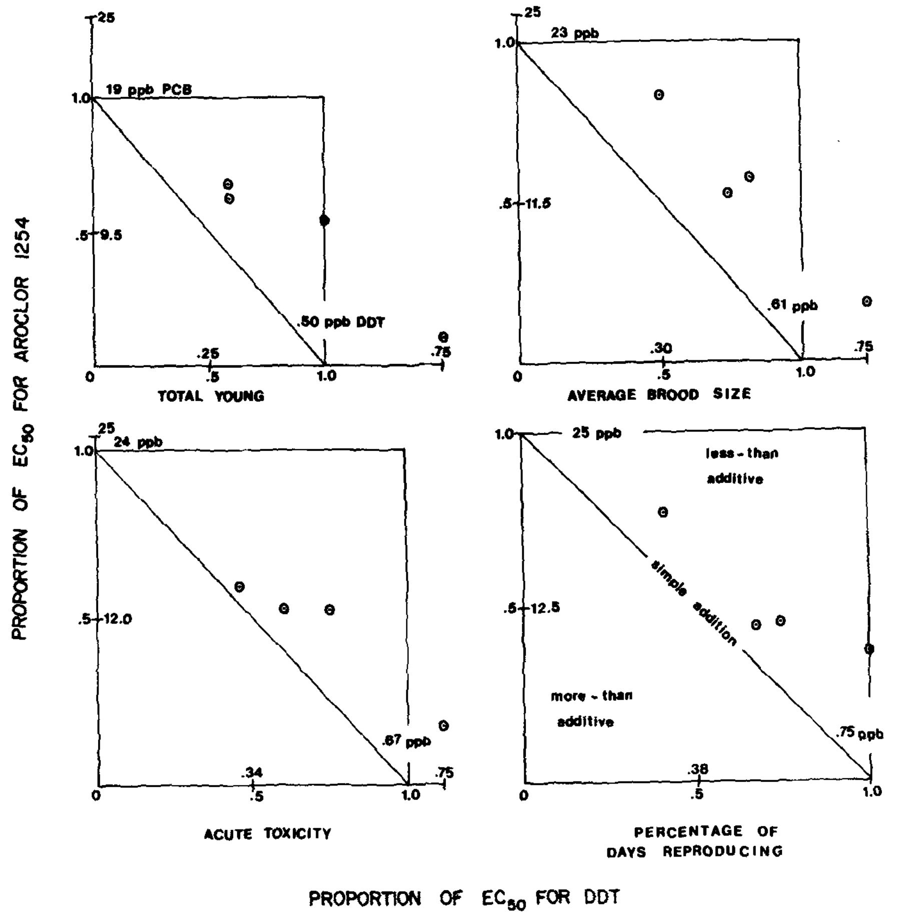

Figure 1. Toxic interaction of DDT and Aroclor 1254 combinations on survival and reproductive inhibition of *Daphnia magna*. Points labelled inside of coordinate axes correspond to parts per billion of toxicant.

action square and demonstrate the interaction of low levels of PCB with the relatively high level of DDT. The toxic effects are severe due to DDT alone since this concentration exceeds the 14-day EC_{50} for DDT.

Discussion

Our experiments indicate that Daphnia magna is an indicator of the toxicity of both DDT and Aroclor 1254 as measured by static toxicity tests. The 14-day LC_{50}'s for DDT and PCB were 0.67 ppb and 24.0 ppb respectively with effective values for reproductive inhibition as low as 0.30 ppb for DDT and 10.0 ppb for Aroclor 1254. The susceptibility of Daphnia cultures was best represented by the count of total young produced in sublethal concentrations of toxicants. For many of the bioassays, the EC_{50} for number of young produced was one-half to two-thirds of the LC_{50} level indicated from acute toxicity data alone. Values for average brood size and acute toxicity also showed generally greater susceptibility than the percentage of days that young were produced.

These results indicate that concentrations of DDT at 0.30 and 0.50 ppb increased the toxicity of Aroclor 1254 to Daphnia by as much as 2X and that exposure to a no-effect concentration of 12 ppb PCB served to increase the susceptibility of Daphnia to DDT by an average of 20% With PCB and DDT concentrations treated as simple effects, a factorial analysis of variance examining the interaction of the toxicants yields a statistically significant interaction term for acute toxicity and reproductive inhibition data. Graphic analysis of these data indicates that the two chemicals are helping one another in joint action and that the interaction existing between the two chemicals is less-than-additive. The toxicants are working together but their combined effect is slightly less than would be predicted by simple additive toxicity.

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