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Potential Influence of Acetone in Aquatic Bioassays Testing the Dynamics and Effects of PCBs[1]

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Most organic chemicals that bioaccumulate in fish are characterized by extremely low water solubility, requiring researchers to rely on solvents such as acetone to solubilize organic contaminants. The validity of this procedure rests on the assumption that the solvent will not affect the results either by itself or through interaction with the test chemical. However, there is little published evidence that even low levels of acetone are harmless (HALTER & JOHNSON 1974). To evaluate the effects of acetone on uptake of PCBs and growth in fish, we conducted a study in which fry of lake trout (Salvelinus namaycush) were exposed to relatively low levels of PCBs in water and food with and without the addition of acetone to the water.

MATERIALS AND METHODS

The experimental design consisted of four treatments: (1) PCBs with acetone, (2) PCBs without acetone, (3) acetone, and (4) neither PCBs nor acetone. Nominal concentrations of PCB were 50 ng/L in water and 1 mg/kg in food for fish receiving PCBs. Acetone concentration was 10 µL/L. The PCB in this report was Aroclor 1254 (Monsanto)[2] and the acetone was pesticide grade. For each treatment, we used two 190-L fiberglass tanks, each containing 650 lake trout fry. Concentrations of PCBs in the fry were measured four times during the study, and the resulting concentrations were compared between treatments. A volume dependent diluter provided a pulsed flow of laboratory well water (total hardness, 465 mg/L; total alkalinity [$CaCO_3$] 330 mg/L; and pH, 7.2) averaging 2 L/min at 9°C to each tank (BERLIN et al. 1981).

Exposure System. For fish exposed to PCBs without acetone, PCBs were dissolved in water by pumping water through a column packed with PCB-coated glass beads. This column consisted of a

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30.5-cm length of 3.8-cm polyvinyl chloride (PVC) pipe packed with 4-mm glass beads and stoppered on each end with glass wool. After rinsing the column with petroleum ether, we added a solution of 4 g PCBs in petroleum ether dropwise to the column until the solvent was observed draining out of the opposite end. We collected the drained solvent, concentrated it, and again added it to the column until all 4 g of PCBs were coated on the column. The column was air dried, fitted with a PVC reducing adapter on each end and connected to a continuously recycling reservoir system incorporating an 18-L glass jar. A constant flow of water through the column to the jar maintained an equilibrium concentration ranging from 17 to 28 µg/L PCB at temperatures ranging from 10 to 21.5°C. Desired water temperature in the jar was attained by using a glass heater and a mercury contact thermoregulator. Water volume in the jar was controlled by a float valve on the water inlet. A pump delivered 40 mL of the PCB solution from the jar to a mixing tank containing a mechanical stirrer. Water was added, stirred, and distributed among the tanks every 2 min, providing a nominal concentration of 50 ng/L. A 2.54-cm solenoid valve controlled the flow from the mixing tank and maintained an average rate of 2 L/min through each tank.

For fish exposed to PCBs with acetone, we added a stock solution of 4.0 µg PCBs/mL of acetone to a mixing tank by using an infusion pump containing three syringes. Each syringe delivered 0.0395 mL per cycle to the mixing tank which also received 8 L of water. The water was mechanically stirred and delivered through a solenoid valve to the two tanks. This system also provided nominal PCB concentrations of 50 ng/L and a nominal acetone concentration of 10 µL/L. Fish exposed to PCBs in water also received food with added PCBs, but fish in control treatments received unaltered food. We used spiked food to evaluate the influence of acetone in a laboratory simulation of field conditions. We prepared spiked food by adding 80 mL of acetone containing 5 mg PCBs to 5000 g of Silver Cup feed. The solution was added dropwise while the food was being mixed. Mixing continued until all solvent had evaporated.

For fish exposed to only acetone, the acetone was added by infusion pumps that delivered 0.06 mL/min to each tank. This provided a nominal concentration of 10 µL/L acetone, the same as the acetone concentration in the exposure tanks containing PCB plus acetone. Control tanks (without acetone or PCBs) received water at 2 L/min.

Fish. Eyed eggs of lake trout were obtained from the Jordan River National Fish Hatchery on December 7. On January 23, when over 90% of the fry had hatched, 10 groups of 25 fry each were randomly sampled for contaminant analysis, and 650 fry were put into each of the eight treatment tanks. Water temperature was 9.0°C and photoperiod was set at 11L:13D; overhead fluorescent lights provided illumination. Half of each tank was covered with black plastic to offer a shaded area and the other half with opalescent fiberglass sheets that allowed passage of diffuse light.

Feeding with Silver Cup salmon starter began when 10 to 20% of the fry were at swim-up (day 22 of exposure). When active feeding by most of the fry in the tanks was observed (day 35), the ration was set at 10% of body weight/day based on the number and average weight of fry in each tank. Fry were fed 3 or 4 times each day.

Analytical Techniques. To monitor the performance of the exposure systems, we analyzed water in each tank twice a week by collecting 1500 mL water and extracting each sample twice with 25 mL of petroleum ether. The extract was placed on silica gel columns and PCBs were eluted from the column with 10 mL of 2% diethyl ether in petroleum ether. The final extracts were evaporated to 0.5 mL in iso-octane and analyzed by gas chromatography, as described by BERLIN et al. (1981).

Samples for analysis of PCBs in tissue were composites of 25 fry. Ten samples taken on day 0 provided pre-exposure PCB concentrations for all treatments. For each of five samples of 25 fry collected from each tank on days 17 and 41, we measured wet weight, freeze-dried weight, and total lengths of the individual fry. We recorded mortalities daily and measured total length of dead fry. The remaining fry in each tank were removed at the end of the study (day 52) and analyzed in groups of 25.

We saponified the samples in 20 mL scintillation vials by adding 15 mL of the saponification mixture (10 g KOH, 6 mL deionized water, and 34 mL ethanol) to each vial and allowing it to digest at about 50°C for 2-3 h. Younger fry required longer digestion times, probably due to the large amount of yolk present. We extracted the saponified samples with two 30 mL volumes of hexane and then added 30 mL of saturated aqueous solution of $(NH_4)_2SO_4$ to the hexane to coprecipitate a colloid that formed in each sample when the hexane was concentrated. This colloid appeared to be proteinaceous and was easily salted out with $(NH_4)_2SO_4$. To make sure that removing the colloid and discarding the precipitate did not affect analytical results, we analyzed 12 replicate samples, 6 by goldfisch extraction with hexane, and 6 by saponification; we found no differences in PCB concentrations. We also saponified food samples, using 10-gram samples of spiked and unaltered Silver Cup salmon starter. Hexane extracts of all saponified samples were concentrated, fractionated on silica gel, evaporated to 1 mL in iso-octane, and quantified by gas chromatography. PCB concentrations in fish tissue were based on dry weight and concentrations in food were based on wet weight (9% water).

Data Analysis. We compared dry weight concentrations on individual sampling days using nested analysis of variance on orthogonal contrasts. Effects of the treatments on mean growth (dry weight) were compared by nested analysis of variance and Duncan's multiple range test. Mean lengths of fry on day 52 were compared by analysis of variance. Mortality data were adjusted for sampling (see BERLIN et al. 1981) and treatment effects were

compared by chi-square tests. We also used chi-square to test differences in the length-frequency distributions between treatments on each sampling day. Null hypotheses were tested using $\alpha = 0.05$. Where actual critical values (χ) could be calculated for rejection or acceptance of the null hypotheses, they were reported.

RESULTS

Experimental Conditions. Nominal concentrations of PCBs in water for tanks receiving PCBs were maintained at 50 ng/L by frequently measuring PCBs in the stock solutions and adjusting temperatures and volumes in the diluter system. Analyses for PCBs in water from exposure tanks yielded values somewhat lower than nominal, the mean concentrations ranging from 40 to 50 ng/L. No significant differences ($P \geq 0.50$) were detected between treatment means of 43 ± 4 ng/L for PCB without acetone and 50 ± 6 ng/L for PCB with acetone over the entire 52-day exposure period.

Mean water temperatures by treatment were 9.1 and 9.0°C for control groups with and without solvent, respectively, and 9.3 and 9.2°C for groups receiving PCBs with and without acetone, respectively. Slightly higher temperatures in treatments receiving PCBs were a function of using the mixing tank. These small temperature differences are unlikely to have contributed substantially to rate of accumulation (REINERT et al. 1974) or growth (McCORMICK et al. 1972).

PCB concentrations averaged 0.72 ± 0.005 µg/g in fortified food samples and 0.06 ± 0.005 µg/g in unaltered food samples.

PCBs in Lake Trout Fry. PCBs were accumulated rapidly by fry receiving PCBs in the water, the initial levels of 0.4 ± 0.02 µg/g ($\bar{x}$±SE) increasing to about 10 times that value in the first 17 days (Table 1). By day 41, concentrations increased to 11.4 ± 1.5 and 10.5 ± 0.4 µg/g in fry from tanks receiving PCB with and without acetone, respectively, but this difference was not significant ($P \geq 0.05$). At the end of the study, concentration of PCBs was 11.9 ± 0.2 µg/g in fry receiving PCBs with acetone--significantly higher ($P \leq 0.05$) than 9.0 ± 0.5 µg/g in fry receiving PCBs without acetone (Table 1). Although the PCB concentration in fry receiving PCBs without acetone dropped significantly ($P \leq 0.02$) from day 41 to day 52, there was no net loss of the contaminant in the fish. Analysis of variance on body burdens (µg PCB/sample) revealed that PCBs in these fry significantly increased ($P \leq .001$) from 5.9 µg/sample on day 41 to 8.4 µg/sample on day 52. Fry receiving PCBs with acetone contained body burdens of 10.6 µg/sample on day 52, which was also significantly different ($P \leq 0.001$) from final burdens in fry receiving PCBs without acetone. Concentrations also increased in fry in control tanks with and without solvent, from 0.4 ± 0.02 to 0.9 ± 0.04 µg/g on day 17 (Table 1), and by day 41, PCB concentrations increased to an average of 1.3 ± 0.1 µg/g in fry from the two sets of control tanks. At the end of the study, PCB concentrations were 1.6 ± 0.3 µg/g in fry receiving acetone alone and 1.2 ± 0.03 µg/g in fry not receiving acetone, however, these concentrations were not significantly different ($P \geq 0.05$).

TABLE 1. Mean dry weight concentrations of PCBs ($\bar{x}$) in micrograms per gram, and standard errors in parentheses for control fry and fry exposed to PCBs with and without solvent.

Sampling day	Treatment			
	Control with solvent	Control without solvent	PCB with solvent	PCB without solvent
Day 0				
$\bar{x}$	0.4 (0.02)	0.4 (0.02)	0.4 (0.02)	0.4 (0.02)
Day 17				
$\bar{x}$	0.9 (0.05)	0.9 (0.04)	3.8 (0.24)	3.8 (0.20)
Day 41				
$\bar{x}$	1.3 (0.19)	1.4 (0.50)	11.4 (1.47)	10.5 (0.38)
Day 52				
$\bar{x}$	1.6 (0.31)	1.2 (0.03)	11.9 (0.24)	9.0 (0.48)

Growth of Lake Trout Fry. No significant differences ($P \geq 0.05$) in mean dry weight of fish existed between treatments until day 41, when fry receiving PCBs with acetone weighed significantly more ($P \leq 0.05$) than fry in the other three treatments. However, no differences in the length frequency distributions were observed ($P \geq 0.05$) between treatments on this day (Fig. 1).

By day 52, dry weights differed significantly between treatments. Fry receiving neither PCBs nor acetone weighed the least (29.1 ± 0.6 mg), followed by fish receiving acetone alone (32.5 ± 0.8 mg), fish receiving PCBs with acetone (35.5 ± 0.5 mg), and fish exposed to PCBs without acetone (37.4 ± 0.7 mg, Table 2). Although Duncan's Multiple Range Test ranked the two control treatments as not significantly different ($P \geq 0.05$), it also ranked the three treatments that received PCBs, acetone, or both as similar ($P \geq 0.05$).

Length frequency distributions were also significantly different between treatments on day 52. That of fry receiving neither PCBs nor acetone was reasonably normal, with a mode at 30-31 mm (Fig. 1), and differed significantly from that of fry receiving PCBs without acetone ($P \leq 0.004$) and only acetone ($P \leq 0.04$). Modes of these last two distributions were at 32-33 mm, indicating growth enhancement by both PCBs and acetone. The distribution of lengths of fry receiving PCBs with acetone, with the mode at 34-35 mm, was significantly different from that of lengths of fry receiving only acetone ($P \leq 0.02$), again indicating growth enhancement due to PCB exposure.

TABLE 2. Mean dry weights ($\bar{x}$) in milligrams, and standard errors in parentheses for control fry and fry exposed to PCBs with and without acetone.

Sampling day	Treatment			
	No solvent control	Solvent control	PCB without solvent	PCB with solvent
Day 0				
$\bar{x}$	26.2(0.6)	26.2(0.6)	26.2(0.6)	26.2(0.6)
Day 17				
$\bar{x}$	20.7(0.3)	20.6(0.3)	20.0(0.2)	21.6(0.2)
Day 41				
$\bar{x}$	21.4(0.5)	21.7(0.3)	22.3(0.4)	23.9(0.8)
Day 52				
$\bar{x}$	29.1(0.6)	32.5(0.8)	37.4(0.7)	35.5(0.5)

Mortality through the 52-day exposure was low--4.9% in fry receiving neither PCBs nor acetone and 6.5% in fry receiving only acetone. Mortality was identical, at 6.3%, in both PCB exposures with and without acetone. Statistical (chi-square) analyses revealed no significant differences ($P \geq 0.05$) in mortality among all four treatments.

DISCUSSION

Fry receiving PCBs with acetone accumulated significantly ($P \leq 0.05$) more PCBs after 52 days than did fry receiving PCBs without acetone, indicating that acetone affected PCB accumulation. However, we did not observe significant differences in PCB concentrations in fish until day 52. Up to day 41, both groups of fry accumulated PCBs rapidly, but from day 41 to 52 little accumulation took place in fry receiving PCBs with acetone, and concentration decreased significantly ($P \leq 0.05$) in fry receiving PCBs alone. This decrease in concentration was not associated with an actual loss of PCBs, as evidenced by the highly significant increase in body burden during this period. We believe that the drop in concentration was due to dilution because of the rapid growth of the fry. Similar results were observed in lake trout fry from Lake Michigan exposed to PCBs (MAC & SEELYE 1981), and in fry of brook trout (Salvelinus fontinalis) exposed to toxaphene (MAYER et al. 1975). Between 15 and 60 days of exposure, the toxaphene concentration in brook trout decreased from 8.3 to 1.8 µg/g while the fish were being continuously exposed to 139 ng/L toxaphene. Since our results showed a significant difference in both the concentration and body burden of PCBs accumulated by fry after 52 days of exposure to PCBs with or without acetone, we conclude that acetone influenced the amount of PCBs accumulated by the fry.

Accumulation of PCBs by lake trout fry from both control treatments to the onset of feeding (day 0-17) is evidence that a low level of PCBs was present in the laboratory water. Only a part of this increase in concentration was the result of a 23% loss in body weight during this period of yolk absorption. Trace concentrations of PCBs (<10 ng/L) have been found in laboratory water, although part of this concentration may be due to the analytical process. The presence of 0.06 ± 0.005 µg/g PCBs in control food could be responsible for some accumulation after day 22, when feeding started.

The two major routes of contaminant uptake in fish are from food and from water. Some evidence indicates that most respiration takes place through the yolk sac in newly hatched fry (BROWN 1957); however, because uptake differences did not appear until after yolk sac absorption, we do not believe that respiration through the yolk influenced PCB accumulation differences in the present study.

Growth rate of fish is known to influence contaminant uptake (NORSTRUM et al. 1976), and we observed some significant differences in the length and weight of fry receiving PCBs or acetone, or both, in this study. Growth was affected most by exposure to PCBs; however, acetone alone may have influenced growth. The trend in dry weights of fry on day 52 (Table 2), along with the differences in length frequency distributions on this sampling day, support this contention. The mean dry weight of fry receiving neither PCBs nor acetone was not significantly less than that of fry receiving acetone: however, these measurements were taken after only 17 days of active feeding. A longer growth period may have magnified these differences. Therefore, the possibility exists that some of the observed effects of acetone on PCB accumulation were due to increased growth and associated change in food intake or conversion efficiency.

The observed differences in PCB accumulation between fry receiving solvent and fry receiving no solvent may be a result of differential uptake through the gills. Accumulation through the gills could be increased by an increased flow of water over the gills (i.e., increased ventilation rate) or by an increase in assimilation efficiency of the gills (NORSTRUM et al. 1976). MAJEWSKI et al. (1978) observed a large increase in ventilation rate of rainbow trout (Salmo gairdneri) exposed to a high concentration of acetone (2,930 mg/L) for 1 hour. In addition, absorption by gills can be affected by differences in the solubility of the contaminant (SCHOOR 1975), and the use of acetone to increase the solubility of PCBs in water may have affected its absorption by the gills and possibly, acetone caused differential solubility of the PCB isomers. A higher percentage of the low chlorine, less persistent isomers may have been present in the water with PCB but without acetone. Thus, several mechanisms may have been responsible for the increased uptake of PCBs by fry when acetone was used as a carrier solvent in this study. Our intent

was not to ascertain the mechanisms by which acetone influences the uptake or effects of PCBs, but rather whether the use of acetone would influence the results and interpretation of chronic, low level, contaminant exposures.

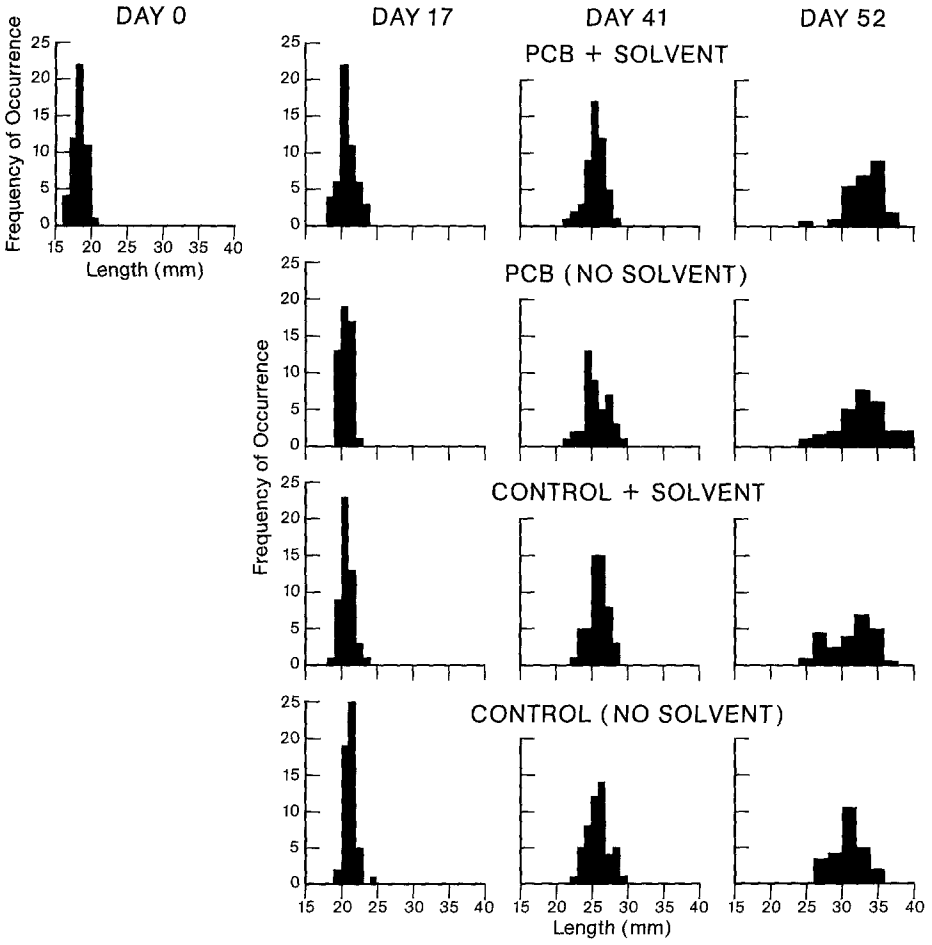

Figure 1. Frequency distributions of lake trout fry lengths (mm) at the beginning of the study (Day 0) and from all four treatments after 17, 41, and 52 days of exposure.

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