Polychlorinated Biphenyl: Interaction with Duck Hepatitis Virus

Abstract. Ten-day-old mallard ducklings fed a polychlorinated biphenyl at concentrations of 25, 50, and 100 parts per million for 10 days suffered no apparent clinical intoxication. Five days later these birds were challenged with duck hepatitis virus, and they suffered significantly higher mortality than birds which were not exposed to the polychlorinated biphenyl.

Polychlorinated biphenyls (PCB) along with DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane] are reported to be the most abundant of the chlorinated hydrocarbon pollutants in the global ecosystem (*1*). Despite the fact that PCB's have been in wide use since 1930 (*2*), they remained ecologically inconspicuous until 1966 when a Swedish chemist reported their presence in the tissues of pike and other wildlife (*3*). Since then, their presence has been reported in additional wildlife of Europe and North America (*1, 4*). These compounds are interesting because they are an important source of interference in the chemical detection of DDT and its metabolites (*5*) and because they are a potential hazard in the environment; on a weight basis PCB preparations have been shown to have an estradiol-degrading potential about five times that of *p,p'*-DDE or technical grade DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] (*1*).

As part of an investigation of possible interactions between organochlorine pollutants and infectious diseases, this study was initiated to determine whether any interaction, antagonistic or synergistic, occurs with PCB and duck hepatitis virus (DHV) in mallards (*Anas platyrhynchos*).

Four separate diets, three with PCB and one without, of a standard duck starter ration were fed to groups of 10-day-old mallard ducklings. The three PCB diets contained Aroclor 1254 (in a corn oil premix) concentrations calculated to be 25, 50, and 100 parts per million (ppm). These diets were fed to five groups of 25 ducklings each, three principal and two control groups (Table 1).

All birds were leg-banded with color-coded, numbered bands, weighed, and fed their respective diets for 10 days. On day 11 each bird was weighed again, and every fifth bird from each treatment group was killed. Tissues were collected for residue analysis, histology, and liver weights. During the 10-day feeding trial, no birds died or became clinically ill, although ducklings in the group given 100 ppm PCB were noticeably hyperexcitable after day 3 as were those in the 50-ppm group 4 days after treatment.

Body weights of birds receiving PCB in their diet were significantly heavier at the end of the 10-day feeding trial than those fed the diet without PCB (Table 1). The heaviest body weights occurred in birds fed the highest concentrations of PCB, and the trend appeared to be linear. Differences in body weights among treatment means were highly significant ($F = 20.30$, d.f. = 4, 119) when tested by analysis of covariance. No statistical comparisons between individual treatment means were made. A similar analysis of liver weights (determined as a percentage of final body weight) disclosed highly significant differences among treatment means ($F = 5.82$, d.f. = 4, 18). These differences appeared to be within groups treated with PCB rather than between treated and untreated groups (Table 1). The small sample sizes ($N = 5$) necessitates caution in interpretation of liver weight data. No interpretation of differences among body and liver weights are offered at this time.

Table 1. Final mean body and liver weights of mallard ducklings after being fed PCB (Aroclor 1254, Monsanto, St. Louis) for 10 days. Numbers in parentheses are the number of birds used. The liver and body weights were adjusted by covariance for a common starting mean; the liver weight was determined as percentage of body weight. The final control did not receive either PCB or DHV.

Treatment	PCB (ppm)	Body weight (g)	Liver weight (g)
PCB + DHV	100	381.6 (25)	0.046 (5)
PCB + DHV	50	368.8 (25)	.037 (5)
PCB + DHV	25	352.8 (25)	.029 (5)
DHV control	0	315.2 (25)	.036 (4)
Control	0	309.6 (25)	.034 (5)

Twelve days after the experiment began, birds were placed in crates and transported approximately 5 miles to isolation units where they were maintained on PCB-free feed and water for 3 days before being challenged with DHV. No birds died or became clinically ill during the interim period between PCB feeding and virus challenge although two birds were lost from the 100-ppm group due to accidents.

On day 15 of the experiment all but the untreated controls (birds not receiving either PCB or DHV) were inoculated intraperitoneally with 1.5 LD_{50} (50 percent effective lethal dose for ducks) of DHV per bird. The first birds died about 47 hours after inoculation. Mortality was recorded hourly for the remainder of the 80-hour experimental period with one exception; there were no observations between hours 63 through 69. The onset of mortality in all groups given PCB plus DHV began at least 8 to 16 hours before that in the control group treated with the virus only (Fig. 1). In addition, mortality levels occurring among PCB-plus-virus treatment groups at the end of the experiment were significantly higher at the .01 level of probability than for ducklings receiving the virus only (chi square = 7.49, d.f. = 1). There was no significant difference in mortality among ducklings receiving the different concentrations of PCB plus virus (chi square = 3.79, d.f. = 2). No birds in the untreated group died (Table 2).

All birds that died during the experiment had gross pathognomonic liver lesions, indicating typical duck hepatitis infection (*6*). No other gross lesions, with the exception of varying amounts of edema within the pericardium and thoracic cavity of some birds, were observed. There did not appear to be any relation between treatment and the presence or absence of edema. Histological and residue analysis have not been completed at this time.

The suggestions that PCB may render a host more susceptible to certain types of infectious agents is the significant

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finding in this study. None of the concentrations of PCB fed resulted in detectable chemical intoxication even with the physical stresses of weighing, handling, confinement, and crowding during transportation, transportation itself, or relocation in a different environment. However, when the stress of an infectious agent was added, these sublethal concentrations appeared to influence the resulting mortality rates, causing two- to fourfold increases (14 percent among virus controls versus 35 to 65 percent among groups receiving PCB plus DHV) and reduced incubation time (Fig. 1 and Table 2).

Similar increases in mortality were obtained with other organochlorine compounds in our laboratory (*7*). In those studies 30-day-old mallard ducklings which had been fed sublethal concentrations of *p,p'*-DDT or dieldrin exhibited three- to ninefold increases in mortality over that of DHV controls (6 percent among virus controls versus 19 to 59 percent among groups receiving dieldrin plus DHV, and 19 to 40 percent among groups receiving *p,p'*-DDT plus DHV). It is unlikely that in all these instances mortality in the DHV control groups would be less than half that in any interaction group on the basis of chance alone.

This study illustrates one of the potentional effects of sublethal concentrations of chemical pollutants which are often alluded to, but rarely documented. It also emphasizes the real differences that exist between "sublethal" and "no-effect" concentrations of pollutants. To the best of our knowledge, other studies in vivo of the possible effects of organochlorine pollutants on the susceptibility of a vertebrate host to a viral agent have not been reported.

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Table 2. Mortality among mallard ducklings caused by DHV during an 80-hour experimental period. No observations were made between 31 to 47 and 62 to 70 hours. The final control did not receive either PCB or DHV.

Treatment	PCB (ppm)	Mortality			
		No. of deaths/ total sample	Percentage	Onset (hours)	Cessation (hours)
PCB + DHV	100	8/18	44.4	31–47	75
PCB + DHV	50	13/20	65.0	55	76
PCB + DHV	25	7/20	35.0	31–47	75
DHV control		3/21	14.3	62–70	73
Control		0/20	0.0		

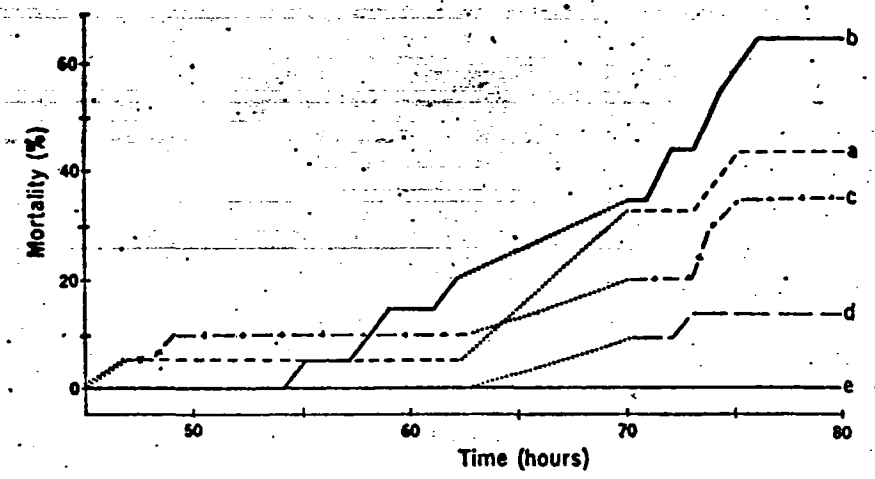

Fig. 1. Mortality among mallard ducklings exposed to both PCB and DHV, DHV only, or neither agent. (*a*) 100 ppm PCB + DHV; (*b*) 50 ppm PCB + DHV; (*c*) 25 ppm PCB + DHV; (*d*) DHV control; and (*e*) untreated control. Dotted portion of lines prior to 47 hours and between 62 and 70 hours represent periods during which no observations were made.

References and Notes

1. R. W. Risebrough, P. Rieche, D. B. Peakall, S. G. Herman, M. N. Kirven, *Nature* 220, 1098 (1968).
2. C. A. Penning, *Ind. Eng. Chem.* 22, 1180 (1930).
3. S. Jansen, *New Sci.* 32, 612 (1966).
4. D. C. Holmes, J. H. Simmons, J. O'G. Tatton, *Nature* 216, 227 (1967); A. V. Holden and K. Marsde, *ibid.*, p. 1274; J. H. Koeman, M. C. Ten Noever De Brauw, R. H. DeVes, *ibid.* 221, 1126 (1969); S. Jensen, A. G. Johnels, M. Olsson, G. Otterlind, *ibid.* 224, 247 (1969).
5. G. Widmark, *J. Ass. Offic. Agr. Chem.* 50, 1069 (1967); L. M. Reynolds, *Bull. Environ. Contam. Toxicol.* 4, 128 (1969).
6. P. P. Levine, in *Diseases of Poultry*, H. E. Beister and L. H. Schwarte, Ed. (Iowa State Univ. Press, Ames, 1965), p. 838.
7. M. Friend and D. O. Trainer, *J. Wildlife Dis.*, in press.
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