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Toxicity Studies on Polychlorinated Biphenyls in the Chick

1. TOXICITY AND SYMPTOMS

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(Received for publication December 10, 1970)

INTRODUCTION

POLYCHLORINATED biphenyls (PCBs) are a series of inert, chemically resistant plasticizers compatible with a wide variety of materials, such as paints, synthetic resins, varnishes and waxes and are used to improve such properties as chemical resistance, water resistance, adhesiveness and flexibility (Monsanto, 1968).

Since PCBs appear extensively in the environment (Risebrough *et al.*, 1968) and because of their chemical properties and widespread use, it appears important to study their potential effects on biological systems.

Some work on the effects of PCBs on chickens has appeared in the literature. McCune *et al.* (1962) observed that an epoxy-resin paint caused symptoms of "chick edema" when chicks were placed in the battery before the paint had hardened. Subsequent investigation revealed that the amine hardener was the cause and that one of the components, a chlorinated biphenyl (Aroclor 1242*), seemed to be the problem compound.

Levels of 100, 200, 400 and 800 p.p.m. of this compound produced labored respiration, abdominal fluids, hydropericardium, and swollen kidneys. The mortality was severe and weight gains reduced.

Further work by this group (Flick *et al.*, 1965) suggested that 400 p.p.m. were required to produce serious symptoms. In addition to the above symptoms, they observed cream-colored pancreas, enlarged adrenal, small spleen, dermatitis and defeathering. Hemoglobin and hematocrit were reduced and blood glucose was reduced slightly.

Koeman *et al.* (1969) reported that 2000 p.p.m. killed 5 out of 5 Japanese quail in less than 2 weeks. Anderson *et al.*

Published with the approval of the Director of the Wisconsin Agricultural Experiment Station College of Avricultural and Life Sciences, Madison.

* Registered trademark for a compound containing 42% chlorine. Sold by Monsanto, St. Louis, Mo.

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(1969) have reported on the PCB content of eggs of pelicans and cormorants obtained in remote areas as well as near industrial areas. Fat extracted from fish in Lake Michigan was examined in this laboratory and contained up to 120 p.p.m. PCBs (Bradley, 1970).

Data on the effect of various levels of PCBs in the diet of the chick could be used to determine safe levels of PCBs in chick feed. The possibility exists that ingredients could contain significant levels of PCBs and that poultry could ingest significant quantities.

Studies on PCB toxicity in the chick could be used in combination with data from trials on other experimental animals to assess the potential hazard of PCB to man.

Data obtained by direct experimentation is of more value than that obtained in indirect ways and the young chick is an easy animal to control for this type of experimentation.

EXPERIMENTAL

Diets containing various levels of PCBs were prepared by adding a PCB (Aroclor 1248**), which contains 48% chlorine, to the basal diet in Table 1. PCBs were dissolved in petroleum ether and mixed into a small portion of the basal diet prior to final mixing to facilitate even distribution throughout the mixture. The basal diet was analyzed using the method of the Food and Drug Administration (1968) and found to contain < 1 p.p.m. of compounds which show on the chromatograph to be similar to PCBs.

Ten one-day-old chicks, resulting from the crossing of New Hampshire males and S.C. White Leghorn females, were placed on diets containing various amounts of PCBs. All experiments were conducted in electrically heated batteries with raised wire floors. Feed and water were provided *ad libitum*. Chicks were weighed weekly, deaths and toxicity symptoms were recorded daily. Seven birds that died on PCB diets were examined by the Wisconsin State Animal Health Laboratory in Madison for gross pathology and indications of toxicity. The investigators also autopsied many of the dead birds. Some of the birds were killed, their livers removed, weighed and reported as a percentage of body weight.

Experiments were terminated at 4½ to 5 weeks. In reporting the results, some of the data were combined and are indicated in the Results and Discussion section.

TABLE 1.—*Basal diet*

	gm./kilogram
Ground yellow corn	525
Wheat middlings	100
Alfalfa meal (220 I.U. A/gm)	50
Meat scrap (50% protein)	50
Soybean meal (44% protein)	200
Fish meal (60% protein)	50
Iodized salt	5
Chick size oyster shell	10
Granite grit	10
	mg./kilogram
$MnSO_4$	136
Vitamin B_{12} (.132 mgs./gm.)	84
Riboflavin (44.3 mgs./gm.)	53
Penicillin (44.3 mgs./gm.)	99
Vitamin A (10000/gm.)	221
Vitamin D_3 (8830/gm.)	99

** Obtained from Monsanto, St. Louis, Mo.

RESULTS AND DISCUSSION

Survivors and average weights of groups of 10 chicks each fed diets containing 0, 10, 50, 100, and 150 p.p.m. PCBs were plotted in Figure 1. A depressed growth rate was observed with dietary levels of 50 p.p.m. PCBs and greater. When 100 p.p.m. and 150 p.p.m. PCBs were added to the diet, toxicity did not increase proportionately. This has also been reported in rats by

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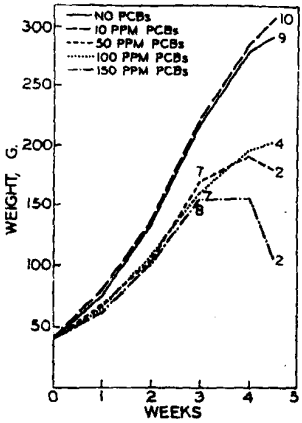

FIG. 1. Survival and average weights from 10 chicks fed various levels of PCBs.

Tucker and Crabtree (1970). Mortality was as severe with 50 p.p.m. as with 3 times as much PCBs in the diet. The survivors are shown directly on Figure 1. All 10 survived unless indicated. Flick *et al.* (1965) reported no deaths when 24 Single Comb White Leghorn cockerels were fed diets containing 200 p.p.m. PCBs (Aroclor 1242) and only 4 deaths in 24 chicks on a diet containing 400 p.p.m. PCBs. Aroclor 1242 is a PCB which contains 6% less chlorine than Aroclor 1248 and presumably this accounts for somewhat lower toxicity. Total mortality with Japanese quail was observed between 6 and 55 days after feeding a diet of 2,000 p.p.m. PCBs (Koeman *et al.*, 1969). The 10 p.p.m. level resulted in small insignificant increase in weight gain especially during the last ½ week.

The next experiment was designed to ascertain how levels below 50 p.p.m. PCBs would affect growth, symptoms and toxicity in the chick. Average weights of chicks fed diets containing levels of PCBs from 10–50 p.p.m. are shown in Figure 2. Three experiments have been averaged together in this figure. In one of the three experiments the 20 and 40 p.p.m. groups were not included in the experimental design. Growth depression resulted from PCBs even at levels where no deaths occurred. There was considerable variation in how individual birds react on the same diet. Two birds on the diet containing 30 p.p.m. PCBs each weighed 208 g. at 3 weeks of age. At 4 weeks of age one had gained 14 g. and the other 105 g. The 40 p.p.m. growth curve was almost identical with the 30 p.p.m. curve. One bird on the diet containing 50 p.p.m. PCBs lost 52 g. between 3 and 4 weeks of age (206–154). One bird on this

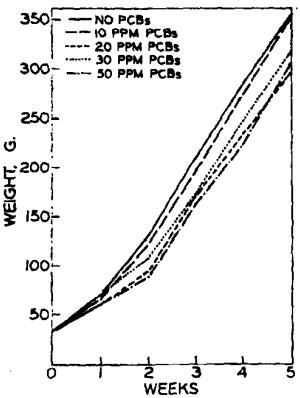

FIG. 2. Average weights of chicks fed various levels of PCBs. (30 chicks started in each group except for the 20 and 40 p.p.m. where only 20 chicks were started.)

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diet gained 10 g. (213-223) and one gained 79 g. (208-287).

Sixteen out of 30 chicks died when 50 p.p.m. PCBs were fed, yet only 4 deaths out of 20 occurred at 40 p.p.m. and 1 death out of 30 at 30 p.p.m. to 5 weeks of age. Levels of more than 10 p.p.m. resulted in a growth depression starting at 1 week of age and continuing through the fifth week when the experiment was terminated.

Mortality results of 5 separate studies with 50 p.p.m. PCBs are shown in Figure 3. At 34 days, 50% of the chicks had died on the diet containing 50 p.p.m. PCBs. The mortality curve was near normal the first 15 days but losses became more severe as the birds increased in age.

General edema was observed in birds fed diets containing > 20 p.p.m. PCBs. The edema was severe enough so that swelling developed below the beaks and adjacent to the wattles in most chicks at 2½ to 3 weeks of age (Figure 4). When chicks which had been fed diets containing 50, 100 and 150 p.p.m. PCBs were examined postmortem, the report from the Wisconsin Animal Health Laboratory indicated extensive generalized edema. Large amounts of serous effusion into the pericardial sacs, muscles, subcutaneous tissues and abdominal cavity were observed. Apparently, the permeability of chick tissues increases in the presence of PCBs. Others have observed edema due to feeding PCBs but not the double chin effect shown here. McCune *et al.* (1962) and Flick *et al.* (1965) reported that edema was observed in chicks fed 400 p.p.m. PCBs in the diet, however, edema was virtually absent from 24 chicks fed 200 p.p.m. PCBs. Species, sex and 6%



FIG. 4. Typical swellings in chicks with PCB induced edema. Note: the swelling below the beak and adjacent to the wattles.

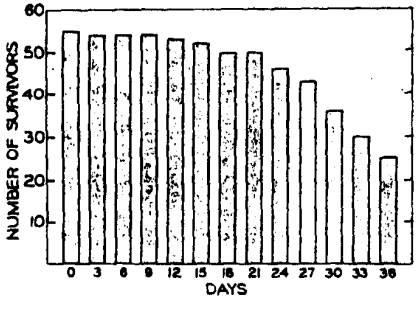

FIG. 3. Survival of chicks on diets containing 50 p.p.m. PCBs. (52 chicks started in 5 studies)

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FIG. 5. Hydropericardium in chick fed a diet containing 50 p.p.m. PCBs.

lower chlorine content of PCBs used in their experiments might account for no or slight edema at 100 PCBs.

An enlarged heart sac is a characteristic symptom of PCB toxicity (McCune *et al.*, 1962; Flick *et al.*, 1965; Koeman *et al.*, 1969). A typical example is shown in Figure 5. In postmortem examination of five 5-week old birds on a diet containing 50 p.p.m. PCBs we removed 10 ml. of fluid from 3 heart sacs, 2 ml. from one, while on the last the heart sac appeared normal. Flick *et al.* (1965) found only 1 ml. (average) when 400 p.p.m. were fed for 3 weeks.

A gasping condition was also observed with high levels of PCBs in the diet. This could be the result of an enlarged heart sac exerting pressure which prevents normal heart action. This would reduce cardiovascular capacity. Moreover, normal lung function would be impaired by the serous fluid and hemorrhages which were observed in postmortem examination.

A noticeable decrease in the development of comb and wattles was observed with chicks fed diets containing 40 and 50 p.p.m. PCBs. Less depression of secondary sexual characteristics was observed at 30 p.p.m. and no noticeable decrease was observed when chicks were fed 10 and 20 p.p.m. PCBs. This effect has not been reported previously for chickens. Risebrough *et al.* (1968) has reported that PCBs injected intramuscularly in pigeons resulted in an increase in estradiol metabolites in the liver 7 days after injection. DDT which is structurally similar to PCBs exhibited hormonal degradation in studies using testosterone and progesterone (Peakall, 1967). Degradation of steroid hormones by hepatic enzymes which were induced by PCBs could account for the depression of secondary sexual characteristics which were observed.

In some birds fed a diet containing 50, 100 or 150 p.p.m. PCBs, a postmortem examination revealed that the proventriculus was atypically solid and firm to the touch. Full and distended crops were observed in some birds fed 50 p.p.m. PCBs in the diet. In one experimental group, 6 out of 10 birds had abnormally distended crops at 2½ weeks of age. This condition lasted only 2 or 3 days and was not observed at the lower levels of dietary PCBs. This indicates a temporary stasis of the digestive tract.

Three deaths which the investigators observed were preceded by a convulsion. Other chlorinated hydrocarbons are known to interfere with the nervous system. DDT attacks the nervous system of animals and disrupts the ion-transport mechanism (Matsumura, 1969). This disruption can result in respiratory failure or secondary poisoning by neurotoxins produced in the animal during DDT poisoning.

Liver as a percentage of body weight increased as PCBs in the diet increased (Table 2). This increase in liver as percentage of body weight was also observed when diets contained chick edema factor (CEF)

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were fed to chicks (Campbell and Friedman, 1966). The data do show, however, that actual liver weights per bird are not affected. The percentage change is caused by the weight reduction.

Postmortem examinations indicated muscular hemorrhaging in some birds which had died on diets containing 50, 100 or 150 p.p.m. PCBs. Lungs contained serous fluid and some hemorrhaging. Some hearts showed petechial hemorrhages. Bleeding from the mouth was observed in many chicks which had died during the experiments reported here.

Many of the symptoms of toxicity such as edema, hemorrhaging and lack of tissue integrity suggest that PCBs interfere with utilization or absorption of fat soluble vitamins.

Symptoms observed in chicks fed PCBs are similar to those reported when diets containing CEF were fed. Symptoms include: 1) depressed body weight, 2) edema, 3) pericardial fluid, 4) increased cell wall permeability and 5) an increase in liver as percentage body weight. The constituent responsible for CEF is known to contain a high proportion of chlorine (Yartzoff *et al.*, 1961). Because of the similarity to symptoms and the high proportion of chlorine in both PCBs and CEF, one might postulate that their mode of action is similar.

Other workers (McCune *et al.*, 1962; Flick *et al.*, 1965) with chickens and Tucker and Crabtree (1970) with ducks reported that higher levels are required for toxic symptoms to develop than were needed in these experiments. Generally, the other workers have not worked with levels below 100 p.p.m. This could account for the differences in the results. The best possibility at this time for the differences is the relative toxicity of PCBs based on the chlorine content. Only the compound containing 48% chlorine was tested in these experiments but chickens certainly are affected by relatively low levels of this compound.

TABLE 2.—*Liver as % body weight on toxic levels of PCBs with 2½ week-old chicks*

PCBs added to diet	Weight of birds	Weight of liver	Liver as % body wt.
p.p.m.	g.	g.	
0	218.	5.8	2.7
0	195	6.7	3.7
50	170	5.8	3.4
150	106	4.8	4.5
150	139	6.2	4.5
150	151	6.8	4.5

SUMMARY

Maximum tolerance of polychlorinated biphenyls containing 48% chlorine in the chick is approximately 50 p.p.m. Symptoms observed in the chick fed polychlorinated biphenyls included 1) depressed weight gain, 2) edema, 3) gasping for breath, 4) hyperpericardial fluid, 5) internal hemorrhaging, 6) depression of secondary sexual characteristics and 7) an increase in liver as percentage of body weight. Several of these symptoms were present when levels of more than 20 p.p.m. of PCBs were fed.

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Fumigation and Reuse of Broiler Litter[1]

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(Received for publication December 11, 1970)

THE periodic scarcity of conventional litter materials is a matter of increasing concern to broiler producers in many parts of the country. In areas where the problem has been especially acute some poultrymen have resorted to reusing the litter for 2 or more broods.

Although Kennard and Chamberlin (1947, 1948a, b, 1949, 1951) have demonstrated the effectiveness of built-up litter pathologists and poultrymen are still concerned about the possibility of perpetuating disease organisms to succeeding broods. The use of a soil fumigant, methyl bromide, by Andrews *et al.* (1943) and Boney (1948) for control of coccidia and intermediate hosts of some internal parasites suggested to Edgar and King (1955) the possibility of sterilizing poultry litter with methyl bromide. In a controlled laboratory study and under "semi-field conditions" they reported that methyl bromide killed *Aspergillus fumigatus* on agar, *Ascaridia galli* eggs and *Eimeria tenella* oocysts in a small amount of water when used at the rate of 1 cc. per quart of space or 1 pound per 100 square feet of floor space.

Klepser *et al.* (1962) fumigated two broiler houses under field conditions at the rate of 1/2 and 1-lb. of methyl bromide per 100 square feet of floor space. Test dishes distributed in the litter showed good control of *E. necatrix* and variable results with *Ascaris suum* ova. They also reported no significant mortality among chicks placed on the fumigated litter within a few hours following termination of the fumigation period. Their preliminary report, however, did not contain data on the subsequent performance of these chicks.

The purpose of this study, therefore, was to determine the effect of methyl bromide fumigation of reused litter on chick growth, feed conversion and mortality.

[1] Journal Series No. 1278 of the Hawaii Agricultural Experiment Station.

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MONS 081649