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Effects of Polychlorinated Biphhenyls in the Ring-Necked Pheasant

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EFFECTS OF POLYCHLORINATED BIPHENYLS IN THE RING-NECKED PHEASANT

BY

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EFFECTS OF POLYCHLORINATED BIPHENYLS IN THE RING-NECKED PHEASANT

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Committee

EFFECTS OF POLYCHLORINATED BIPHENYLS IN THE RING-NECKED PHEASANT
Abstract

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Under the supervision of Professor C. Wendell Carlson
and Associate Professor Raymond L. Linder

In 1970 and 1971, cock pheasants (Phasianus colchicus) were given single capsules weekly containing 0 and 25 mg polychlorinated biphenyls (PCB), Aroclor 1254, and hens were given 0, 12.5, and 50 mg. Egg production was reduced ($P < 0.01$) in the hens given 50 mg PCB both years. Fertility, determined by visual inspection of incubated eggs, was unaffected by PCB. Hatchability was lowest in the group of hens given 50 mg in both years but significantly lower ($P < 0.01$) only in 1971. PCB adversely affected the viability of the embryo both years, as the number of eggs pipped but not hatched in PCB groups was higher ($P < 0.05$). PCB did not affect eggshell thickness.

Behavior on the visual cliff shortly after hatching was affected among offspring of hens given 50 mg PCB in 1970 ($P < 0.01$). Offspring of hens given 50 mg chose the visually-deep side of the cliff. No differences in behavior on the visual cliff were evident in 1971. Response to hand catching of offspring was affected in 1970 ($P < 0.01$). The ability of penned pheasants to avoid hand capture was least where both parents received PCB, followed by those where hens only, cocks only, and neither parent received PCB. No differences in response to hand capture among groups were found in 1971. Weights of chicks from hens on 50 mg were lower ($P < 0.01$) at 6 weeks of age in

1970. This effect was not seen in 1971. Survival of chicks in brooders to 6 weeks of age was lower ($P < 0.01$) in offspring of hens given 50 mg PCB weekly in 1970. No differences were found among offspring of treatment groups in 1971. Survival from 6 weeks of age to fall was similar in all groups. Overall survival from hatching to fall was less ($P < 0.05$) in offspring of hens given 50 mg weekly in both years combined. No differential effect was found between sexes for survival.

PCB in doses varying from 10 mg to 210 mg daily caused hyper-excitability, weakness, tremoring, lack of appetite, lack of feathers, and a comatose death. Birds 11-weeks old given 10 or 20 mg PCB daily, birds 6 to 9 months old given 50 or 100 mg each 3.5 days, and adult hens given up to 50 mg once a week continued to eat. Eleven-week old birds given 210 mg daily stopped eating. Of those given 210 mg daily, the heaviest birds lost the greatest percentage of their weight (15 to 31 percent) before death occurred (1.3 to 4.3 days). Birds intentionally starved at the same time lost a greater percentage of weight (27 to 51 percent) before death (2.3 to 8.6 days).

Some adult hens were given a single 50-mg capsule and samples of whole body, brain, liver, muscle, eggs, and feces were analyzed to determine patterns of storage and excretion. Levels of PCB were highest at 12 hours after capsule administration. Residues were highest in liver, followed by brain and muscle over 28 days following the single 50-mg capsule. From 94 to 98 percent of the administered dose was absorbed and 40.5 mg of the 50 mg were in the body after 28

days. Hens were in a state of low egg production and excreted 4.2 mg in the eggs and 4.0 mg in the feces over 28 days. Excretion in the egg could be an important means of ridding the body of PCB. Four hens from the 1970 breeding experiment, given 17 capsules weekly containing 12.5 mg PCB and killed 1 week following the last capsule, had from 37 to 56 percent of the administered dose in their bodies. Four hens on the 50-mg level in 1970 had from 60 to 82 percent of the dose given. Five hens given 12.5 mg weekly for 16 weeks in 1971 had an average of 23.8 ppm PCB in their bodies 1 week after the last capsule, three hens had an average of 13.6 ppm after 3 months on a clean diet, and three others had an average of 20.9 ppm after 6 months. Excretion was variable and slow.

An analysis of brain, liver, and muscle tissues from dead and surviving birds fed PCB at various levels showed that brain residue levels from 300 to 400 ppm wet weight were indicative of death from PCB toxicosis. Liver and muscle levels were too variable to be useful in assessing cause of death.

Administration of PCB decreased weights of heart and spleen ($P<0.01$) at all levels given and increased weights of kidneys and livers in birds given 10- and 20-mg doses daily ($P<0.01$) but not in birds given 210 mg daily. Splenic atrophy, where spleens were small, pale-tan color, and had wrinkled capsules resulting from depletion of lymphatic nodules, was characteristic of birds given PCB.

PCB and dieldrin were given singly and jointly, and only additive, not synergistic, effects were noted.

Livers of wild pheasants from Pennsylvania, Indiana, and South Dakota had no more than 2 ppm PCB. This indicates low-level contamination.

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Dr. E. J. Bicknell and Dr. Robert J. Bury, Veterinary Science Department, performed necropsies of breeding pheasants that died. Dr. Bury additionally performed necropsies and did histopathology cooperatively which resulted in a joint publication on one aspect of the PCB study. Dr. Robert E. White and Dr. Russell F. Reidinger, Jr., Denver Wildlife Research Center, Bureau of Sport Fisheries and Wildlife, conducted analyses of pheasant samples for PCB residues. Dr. Reidinger cooperated in a joint publication on tissue residue levels associated

with PCB toxicosis. Dr. Yvonne A. Greichus, South Dakota Experiment Station Biochemistry Department, conducted analyses of pheasants given PCB and cooperated in a joint publication on storage and excretion of PCB in the pheasant.

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Livers from wild pheasants were collected by Fred E. Hartman and John J. Kriz, Pennsylvania Game Commission and Robert D. Feldt, Indiana Department of Natural Resources.

Mr. Boyd J. Bonzer, Extension Poultryman, provided help with pullorum testing and ectoparasite control work. Dr. Wayne L. Berndt, Extension Pesticide Specialist, aided with advice and by spraying pens for control of ectoparasites. Mr. David Suter of the South Dakota Pheasant Company at Canton was very helpful in supplying experimental birds. Mr. Carl Ost, Foreman at the Wildlife Research Area, gave freely of his time and helped in caring for birds and maintaining and improving the holding facilities. Mr. Curtis E. Holmquist and Mr. Edmund Guenthner at the Poultry Research Center were helpful with their time and material help. The Monsanto Company supplied the Aroclor product used in this study and Shell Chemical Company supplied the technical grade dieldrin used in the synergism study.

A study of this magnitude requires the help and encouragement of many experts and friendly helpers in many departments, within and

without the University. I am indebted for this help. An essential to the study, but in a different way, has been the support of my family and my wife, Carmen, for which I am grateful.

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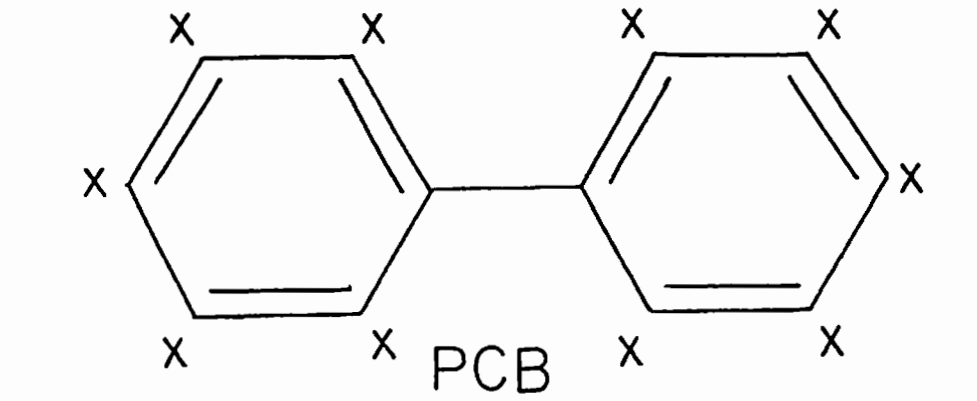
INTRODUCTION

Polychlorinated biphenyls (PCB) have been useful industrial products since the beginning of their commercial production in 1929. They are chemically stable, consisting of two benzene rings with chlorines attached (Fig. 1). PCB are a mixture of compounds and are sold under the Aroclor trade name by the Monsanto Company in the United States. The degree of chlorination gives them differing characteristics and is indicated in the trade name number by which they are sold. For example, Aroclor 1254 is approximately 54 percent chlorinated and is a heavy fluid, while Aroclor 1260 is 60 percent chlorinated and is resinous. They have been used in many common products, such as rubber, floor tile, automobile body sealers, paint, varnish, wax, asphalt, adhesive, polyvinyls, resins, polystyrene, detergents, cardboard cartons, hydraulic oil, cutting oil, electrical products, brake linings, printing ink, and in insecticide formulations. Figures on production and to what extent manufacturers have followed the broad range of uses suggested for PCB by Monsanto in the past are not available.

The first report of PCB occurrence in the environment was made from the work of Sören Jensen, a Swedish chemist (The New Scientist 1966). Since then, PCB have been reported to be widespread in the world's ecosystem, building up in food chains as has been reported for organochlorine insecticides. The chemical structure of PCB is similar to that of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and



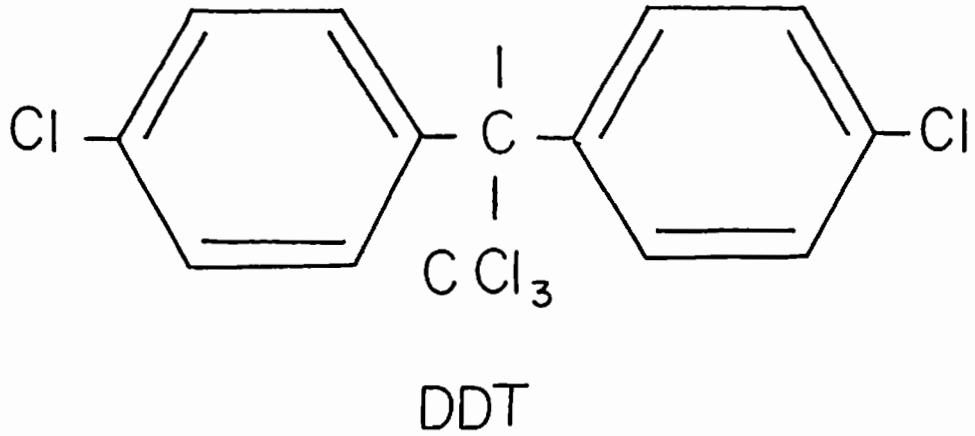


Fig. 1. Structure of PCB compared to that of DDT; x's mark the possible positions where Cl may be attached to the PCB molecule.

their action on organisms, although less toxic, are similar to that of DDT. The contamination of human milk, poultry feed, and all classes of wild vertebrates has created an immediate concern for the need to determine possible harmful effects of PCB.

Since the pheasant (Phasianus colchicus) is of economic importance to the state of South Dakota, as are other gallinaceous birds, it was chosen as the experimental animal for this study. The objectives were to determine (1) the patterns of absorption, storage, and excretion for PCB and (2) the effects of PCB on reproduction, behavior, and survival. Since many published reports indicated that the chromatograms of PCB in wildlife most nearly resemble those of Aroclor 1254, this was the PCB product chosen for study.

REVIEW OF LITERATURE

Scientists in several laboratories, doing gas chromatography for detection of organochlorine insecticide residues, noticed that interfering chemicals were giving peaks at the same positions as DDT, dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene), and other compounds (The New Scientist 1966, Holden and Marsden 1967, Risebrough et al. 1968a). Sören Jensen, a Swedish chemist, determined these interfering compounds to be PCB (The New Scientist 1966). Chemical analyses for PCB in the presence of organochlorine insecticides have presented problems, but techniques for separation and quantitation have been worked out (Risebrough et al. 1969, Koeman et al. 1969, Ahling and Jensen 1970, Westöö and Norén 1970a, Mulhern et al. 1971, Reynolds 1971).

Since Jensen's initial discovery, PCB have been detected in a wide variety of wildlife species in Europe (The New Scientist 1966, Holden and Marsden 1967, Holmes et al. 1967, Widmark 1967, Jensen et al. 1969, Koeman et al. 1969, Prestt et al. 1970) and in the United States and Canada (Holden and Marsden 1967, Risebrough et al. 1968a, Anderson et al. 1969, Bagley et al. 1970, Reynolds 1971). Escape to the environment has occurred since the first commercial production of PCB about 1929 and the description of their properties by Penning (1930).

The PCB found in wildlife have occurred in greatest concentration near areas of industrialization (Holden and Marsden 1967, Jensen

et al. 1969, Risebrough 1970). PCB contamination in the marine environment has been traced to sewage by Holden (1970) in Britain and by Koeman et al. (1969) in the Netherlands. Duke et al. (1970) found PCB in the biota, sediment, and water of an estuarine area, Escambia Bay, in Florida. Nimmo et al. (1971) found that pink shrimp (Penaeus duorarum) and fiddler crab (Uca pugilator) took PCB from the contaminated sediment of Escambia Bay and stored it in their bodies. Hansen et al. (1971) found that pinfish (Lagodon rhomboides) and spot (Leisostomus xanthuras) removed PCB from water for storage in the body. Wildish and Zitko (1971) reported that Gammarus oceanicus absorbed PCB from sea water through their integument. Veith (1970) showed water transport from sanitary and industrial wastes to be a source of environmental contamination in Wisconsin. PCB have also been found in rain water (Tarrant and Tatton 1968) and in snow in Sweden (Smithsonian Institution 1971), although Risebrough et al. (1968b) were unable to demonstrate them in air samples. The New Scientist (1966), Holmes et al. (1967), Widmark (1967), and Biros et al. (1970) reported PCB in human tissue. In Sweden, it was reported by Westöö et al. (1970) that human foodstuffs and human milk were contaminated. Westöö and Norén (1970b) reported fish in Swedish waters to be contaminated.

Fries et al. (1971) found PCB contamination in dairy cattle (Bos taurus) caused by silo paint, and Platonow et al. (1971) found residues in cattle drinking from a contaminated stream in Ontario. Bailey et al. (1970) found that cardboard containers could be a common source of contamination. Another source of contamination

suggested by Reynolds (1969) was the use of PCB to prolong the residual life of insecticides (Hornstein and Sullivan 1953, Sullivan and Hornstein 1953, Tsao et al. 1953, Duda 1957). Lichtenstein et al. (1969) found that PCB increased the toxicity of dieldrin and DDT to insects.

The toxicity of the PCB compounds appears to be related to their relative degree of chlorination, fish and birds metabolizing the more lowly-chlorinated hydrocarbons faster than those more highly chlorinated (Widmark 1967, Jensen et al. 1969, Risebrough 1970). Koeman et al. (1969), through experiments with Japanese quail (Coturnix coturnix), also concluded that the more lowly-chlorinated PCB were metabolized in the bird. Apparently the reverse is true with insects, however, as Lichtenstein et al. (1969) found greater toxicity of the lowly-chlorinated PCB to Drosophila melanogaster and the house-fly (Musca domestica).

Heath et al. (1970) found that pheasants were relatively sensitive to several PCB compounds, ranging from approximately 32 to 62 percent chlorination. These authors ranked bobwhite quail (Colinus virginianus) as most sensitive, followed by pheasants, mallards (Anas platyrynchos), and Japanese quail. They gave mallards 25 ppm Aroclor from 11 weeks prior to the first reproductive season through the second and also fed 50 ppm PCB and 25 ppm PCB jointly with 15 ppm DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] to bobwhite quail in one reproductive season to study reproductive parameters. No differences were found between control and treated birds.

Scott et al. (1971) fed laying chickens (Gallus gallus) Aroclor 1248 at 0.5, 1.0, 10.0, and 20.0 ppm in the diet and found the two higher levels reduced egg production, hatchability, and viability of the embryo at 21 days. Survival and feed consumption were unaffected. Efforts to reduce tissue levels by varying low-energy and high-energy diets were unsuccessful.

Tests at the Patuxent Wildlife Research Center (Dustman et al. 1971) have shown that DDE and Aroclor 1254 were similar in toxicity to grackles (Quiscalus quiscula), cowbirds (Molothrus ster), starlings (Sturnis vulgaris), and redwings (Agelaius phoeniceus). Peakall and Lincer (1970) compared the results of studies by Vos and Koeman (1970) and Stickel et al. (1966) and concluded PCB was probably 1/4 to 1/5 as toxic as DDT. Prestt et al. (1970) concluded PCB to be 1/13 as toxic as DDT. Lichtenstein et al. (1969), working with houseflies, found that PCB were much less toxic than DDT. McLaughlin et al. (1963) found that as little as 10 mg PCB injected into the yolk sac of chicken embryos caused 95 percent mortality and teratogenic effects. Rehfeld (1971) and Rehfeld et al. (1971) studied the effects of PCB on the growing chick and found effects on weight, internal organs, and blood parameters.

The induction of steroid enzymes in the liver by PCB has been demonstrated in both laboratory rats (Rattus rattus) by Street et al. (1969), in pigeons (Columba livia) by Risebrough et al. (1968a), and in the kestrel (Falco sparverius) by Lincer and Peakall (1970). Since the capacity of PCB to induce these enzymes is as potent or more

potent than DDT, PCB have been implicated in eggshell thinning (Risebrough 1970, Peakall 1970, Lincer and Peakall 1970). However, Peakall (1971) found no effect on eggshell thickness from administration of Aroclor 1254 to ring doves (Streptopelia risoria). Bitman and Cecil (1970) found that PCB have slight estrogenic activity when Aroclors with 48 percent or less chlorination were used. Platonow and Funnell (1971) found PCB increased liver weight and decreased body and testes weight and comb size in cockerels and appeared to have an anti-androgenic effect. Villeneuve et al. (1971) found that PCB increased liver weight in the rabbit (Oryctolagus cuniculus) but could not demonstrate increased steroid hydroxylation from PCB administration. Ulfstrand et al. (1971) found migratory activity to be heightened in robins (Erithacus rubecula) given PCB and postulated that a quantitative change in migratory activity could be of considerable importance to the welfare of the robin.

Sax (1963) indicated the liver of man could be injured by PCB. It had earlier been shown that acne resulted from use of products containing PCB (Schwartz and Barlow 1942, Schwartz 1943, Meigs et al. 1954). Contamination of rice bran oil with Kanechlor 400 (48 percent chlorinated) caused Yusho disease in many Japanese users. Studies of acne in Yusho are discussed by Goto and Higuchi (1969).

Liver damage by PCB was demonstrated in guinea pigs (Cavia porcellus), rats, and rabbits by Miller (1944). McCune et al. (1962) and Flick et al. (1965) found that PCB fed to young chickens produced edema and pathological changes in internal organs. Grant et al. (1971)

found increases in organ weights, notably liver, from giving Aroclor 1254 to the rat. Prestt et al. (1970) found that kidneys were larger in the Bengalese finches (Lonchura striata) that died from PCB than in control birds. Vos and Koeman (1970) found that Phenoclor DP 6 and Clophen A60, both of which were manufactured in Europe, caused much more liver necrosis and hydropericardium in chicks than Aroclor 1260. The greater toxicity of the European products was probably due to contaminants (Vos et al. 1970). Friend and Trainer (1970) found that mallard ducklings given PCB were less resistant to duck hepatitis virus and had significantly higher mortality.

Excellent reviews of the history of PCB and the status of our present knowledge about them are given by Risebrough (1970), Peakall and Lincer (1970), and Dustman et al. (1971). Four papers have resulted from the present PCB study concerning effects on reproduction (Dahlgren and Linder 1971), storage and excretion (Dahlgren et al. 1971), residue levels and histopathology associated with acute toxicity (Dahlgren et al. 1972a), and a review of work reported in this thesis (Dahlgren et al. 1972b).

MATERIALS AND METHODS

Aroclor 1254 was mixed (w/w) in a 1:9 dilution with corn oil and weighed into No. 00 size gelatin capsules to within ± 2.5 percent of the required weight. Capsules were administered into the esophagus using a glass tube. Capsules containing only corn oil were administered to control birds.

Breeding, Behavior, and Survival Studies

Hens for breeding experiments were purchased in the winters of 1970 and 1971 from the South Dakota Pheasant Company of Canton (30 and 34, respectively), and cocks were raised from birds previously obtained from the Canton source. All birds used for breeding were about 1 year of age. In late January they were placed under 16 hours daily of artificial light to stimulate breeding. Both sexes were fed a pheasant breeder ration (Zip Feed Mills, Sioux Falls). One tablespoonful of oyster shells was added once each week to the food cup of each hen. Cocks were kept in breeder cages (30 x 36 x 18 inches high) and hens were kept in individual cages (12 x 18 x 12 inches high) designed to facilitate handling and identification of eggs. Eggs were collected daily, individually numbered, set weekly for 15 weeks in a forced-draft incubator, and hatched in pedigree cages.

In 1970, two groups of five cocks each were given either a placebo capsule or one containing 25 mg PCB each week for 17 weeks. Each cock group was mated with three groups of five hens, in which each hen was given a single capsule containing 0, 12.5, or 50 mg

PCB each week for 17 weeks. Resultant offspring were denoted by doses given to parents, first to cocks and then to hens, as 0-0, 0-12.5, 0-50, 25-0, 25-12.5, and 25-50. The same regimen was followed in 1971 except that 14 cocks were used and four additional hens were placed in the group where hens were given 12.5 mg weekly and mated to cocks given 25 mg weekly.

In 1970, cocks were first dosed with PCB on February 13 and weekly thereafter. Hens were first dosed 1 week later, February 20. Breeding was commenced February 16. Eggs were gathered for the first weekly egg group from March 7 to 13 and gathered last 15 weeks later, June 13 to 19. In 1971, the same sequence was followed except that the first dosing began February 19 for cocks and February 25 for hens and breeding began March 1. Eggs were gathered for 15 weekly egg groups beginning March 6 and ending June 18. Weights to the nearest gram were taken of all adult breeders weekly.

Within 36 hours after hatching, chicks were taken from the incubator, wingbanded, and one wing was removed to the alula. They were then placed on a visual cliff (Tallarico and Farrell 1964) and given 5 minutes to jump to either the visually deep or shallow side. Chicks from this study and those hatched from a concurrent dieldrin study were placed together in brooders and fed a turkey pre-starter diet (Zip Feed Mills) until they were 6 weeks of age. At 6 weeks, they were weighed to the nearest gram, placed outside in 16 x 16 foot pens, and fed a pheasant grower ration (Zip Feed Mills).

Several times in summer and fall, chicks were caught by hand by the same person and wingband numbers were recorded. The number of birds in each category caught, in the first and last half of all birds caught, was compared with an expected 50:50 distribution using chi-square analysis. Each pen was a closed unit, and birds of several groups caught predominantly in the first half caused birds of other categories to be caught in the last half.

In 1970, thickness of shells of hatched eggs from all 15 weekly egg groups was measured, without the membrane, to the nearest 0.01 mm, using a micrometer at three points around the mid-line of the egg perpendicular to the long axis. Unhatched eggs from weekly egg groups 8 to 15 were broken, dried for 24 hours, and the shell thickness, including the membrane, measured. Fertility of unhatched eggs was based on a visual inspection of opened eggs for evidence of embryonic development.

Absorption, Storage, and Excretion Studies

Data on storage and excretion of PCB were obtained in four ways: (1) from five groups of four hens each given a single capsule containing 50 mg PCB and checked for residues in eggs, feces, and whole bodies; (2) from whole body analyses of eight hens given capsules containing 12.5 or 50 mg PCB once a week for 17 weeks in the 1970 breeding experiment; (3) from two adult hens given single capsules containing 50 mg PCB and checked for residues in the feces; and (4) from whole bodies of five hens killed 1 week after the last

capsule containing 12.5 mg PCB was given in the 1971 breeding experiment, three hens of the same group killed after 3 months on a clean diet, and three others killed after 6 months.

In No. 1 above, hen pheasants used as controls in the 1970 breeding experiment were randomized into five groups of four birds each. These hens were in a state of low egg production. They were caged separately and fed a commercial breeder ration. One control group of four birds was killed at the beginning of the experiment and pooled for whole-body analysis. A second group of four hens was killed 28 days after treatment and a pooled whole-body analysis was run. Feces and eggs were gathered from hens in the group and pooled weekly. The three remaining groups of four hens each were killed at 12 hours, 1 day, and 7 days after capsule administration. The entire brain and liver of each bird and about 1 inch of pectoralis minor muscle at the posterior end of the sternum were excised, pooled, weighed to the nearest 0.1 mg, and frozen until analyzed.

In No. 2 above, hens from the breeding studies were killed 1 week after administration of the last capsule.

In No. 3 above, feces were collected for 24 hours after capsule administration and at the end of each week for 4 weeks. The hens did not lay eggs.

In order to determine the efficiency of extraction and purification of metabolically-incorporated PCB, six hen pheasants were divided into three groups of two each and confined in separate glass containers. Birds in each group received either 2, 20, or 200 ppm

(based on live weight) of PCB in 80 percent ethanol injected into the breast muscle. Birds were sacrificed after 24 hours during which time no eggs were laid. The entire fresh-frozen body, including feathers and all feces, was finely ground and three 5-gram aliquots from each bird were analyzed.

Residue analyses on the birds in No. 1, 2, and 3 above were done by Dr. Yvonne A. Greichus, Experiment Station Biochemistry Department, South Dakota State University. One-gram samples of brain, muscle, and liver and 5-gram samples of whole bodies, eggs, and feces were analyzed for PCB. Dry weights were obtained by placing the sample in a forced-air oven for 18 hours at 60 C. Lipid determinations employed AOAC (Horwitz 1965:258) procedures. PCB were extracted and purified from the lipid-extracted or fresh samples ground in anhydrous sodium sulfate, using, with modifications, the Florisil column method of Reynolds (1969). PCB were eluted from the column with 200 ml of n-hexane and organochlorine insecticides with 400 ml of 30/70 (v/v) dichloromethane and petroleum ether. However, since these birds had negligible amounts of insecticides, only a single fraction was collected.

All solvents were nanograde. The Florisil was 60/100 mesh, activated at 650 C. It was heated at 130 C for 16 hours, thoroughly mixed with 1 percent distilled water, and stored in an airtight container.

The instrument used for gas-chromatography analysis was a Wilkens Aerograph HY-FI model 600 D equipped with a model S-R 1 mv

Sargent recorder and an electron capture detector cell with a 250-millicurie tritium source. Injector, column, and detector temperatures were 210 C, 190 C, and 210 C, respectively. A borosilicate glass column, 1/8-inch outside diameter by 5-foot, packed with a 1:1 mixture of 15 percent QF-1 Silicone (Fluoro) and 10 percent DC-200 Silicone on 80/100 mesh Chromosorb W (H/P A.W. DMCS) was operated with a flow rate of 40 ml per minute nitrogen carrier gas.

Quantitative determinations were made from the chromatograms by comparing the sums of heights of seven of the peaks of the unknown to the same peaks of a standard solution of Aroclor 1254. All samples were diluted so that the amount of unknown sample injected into the gas chromatograph produced peaks as similar as possible in size to those of the standard.

While accuracy of the PCB determination in ppm is restricted to two significant digits, some of the results are reported to three digits to provide for consistency in tables and for the use of the data in calculations. Duplicate analyses were run on all tissues and values are the average of two runs. Data are shown as ppm wet weight except for feces shown as ppm dry weight.

In No. 4 above, hens were checked for whole-body residues by the Denver Wildlife Research Center. Their analytical techniques will be described in the section following.

Residue Levels and Histopathological Effects

Capsules containing 210 mg PCB were given daily to 11-week-old pheasants randomized into three treatment groups: (1) 16 to be analyzed for residue levels after death, (2) 16 for histopathology upon death, and (3) 39 to be drawn from at time intervals for analysis as survivors for matching with the first 16 mentioned. Corn-oil placebo capsules were given to 14 control birds. These were killed 4.5 days after the first capsule was given. Eleven birds, given no capsules, were starved to be used for histopathology.

Capsules containing 0, 10, and 20 mg PCB were given to three groups of 11 each of 11-week-old birds. Tissues from these pheasants were used for residue analysis and/or histopathology. Control birds were killed 116 days after the first capsule.

Brains and livers were also taken from birds on 50 mg and 100 mg in an experiment that will be described later under "PCB in Combination With Dieldrin." Pooled tissues were analyzed for PCB residues from three cocks and one hen per PCB level from both dead and surviving birds, except that only two dead cocks and one dead hen were pooled and analyzed from the group given 50 mg.

Tissues were analyzed in the laboratory at the Denver Wildlife Research Center as follows. If the sample weighed over 25 grams, a 25-gram aliquot was taken. This aliquot was mixed with five times its weight of sodium sulfate until homogeneous. An amount equal to 15 grams of the sample was placed in a jar with aluminum covering and frozen until extraction. If the sample weighed less than 25 grams

but more than 15 grams, the entire sample was mixed with five times its weight of sodium sulfate and a 15-gram sample was frozen until extraction. If the sample weighed 15 grams or less, the entire sample was mixed with five times its weight of sodium sulfate and extracted.

Extraction was done in a Soxhlet extractor for 6 hours with 225 ml petroleum ether and 25 ml ethyl ether. The extract solution was then transferred to a tared evaporating dish and evaporated to dryness. At this time, lipid weight was obtained. The lipid was then transferred to a 16-mm x 100-mm culture tube and brought up to a 10-ml solution using n-hexane.

Clean-up was done using a column containing 5 grams Florisil deactivated with 5 percent water (w/w) and preconditioned with 50 ml of 3:1 hexane-benzene. A portion of the lipid solution not exceeding 0.05 gram was added and residues were eluted with 125 ml of 3:1 hexane-benzene. The eluate was steam evaporated in a Kuderna-Danish evaporative concentrator with ampoule to less than 10 ml but not to dryness preceding gas-chromatographic analysis.

Gas-chromatographic analysis was done using two EC Ni-63 detectors on a Tracor MT-220 instrument operated at 225 C inlet, 190 C column, and 265 C detector temperatures. Two 6-foot, 0.25-inch outside diameter columns were employed. A 3 percent OV-1, 80-100 mesh on HP Chromosorb W column was utilized for quantitation of Aroclor 1254 and a 5 percent QF-1, 100-120 mesh on HP Chromosorb W column was utilized for confirmation. Nitrogen was used as carrier

gas and maintained at 90 cc per minute through the OV-1 column and 60 cc per minute through the QF-1 column. The sample Aroclor 1254 was identified and confirmed by comparison with the profile of a 1 ppm Aroclor 1254 reference. Reference and sample injection quantities were matched (1 microliter using a 10 microliter syringe) and recorder profile areas were approximately matched by sample dilution with isooctane to avoid error from nonlinear detector response. The area under the sample profile was compared with the area under the reference profile using a compensating polar planimeter to determine the quantity of PCB present. Since no extraneous peaks were observed in the samples, separation procedures were not necessary.

Data were taken from single analyses, as several duplicate runs showed close agreement. The recovery of Aroclor 1254 has been found to be 89 ± 10 percent standard deviation in previous work at the Denver Wildlife Research Center. Data are shown as ppm wet weight to two significant digits.

Birds dying during the study were refrigerated until necropsy examination within 12 hours of death by Dr. Robert J. Bury, Department of Veterinary Science, South Dakota State University. Gross lesions were noted and weights of liver, spleen, heart, and kidneys were recorded to the nearest 0.01 gram. Lung, heart, spleen, liver, kidney, proventriculus, gizzard, adrenal, ovary, pancreas, duodenum, and brain were fixed in 10-percent neutral, buffered formalin. Tissues were dehydrated through increasing concentrations of alcohol and xylene and were embedded in paraffin. Sections 6

microns thick were stained with hematoxylin and eosin and, in some cases, Congo red.

PCB in Combination With Dieldrin

Both cocks and hens from 6 to 9 months old were stratified by sex and weight and randomized to five groups of 22 birds each. They were given capsules containing either (1) 4 mg technical grade dieldrin, (2) 8 mg dieldrin, (3) 50 mg PCB, (4) 100 mg PCB, or (5) 50 mg PCB and 4 mg dieldrin together. Ten capsules were given, one each 3.5 days for 5 weeks. Three and one-half days after the last capsule was given, all surviving birds were sacrificed.

Residues in Wild Birds

The Denver Wildlife Research Center analyzed for residues of PCB in pooled livers from three road-killed pheasants collected near Washington, Pennsylvania; six road-killed pheasants collected southeast of Lancaster, Pennsylvania; six pheasants shot east of Gary, Indiana; six pheasants shot in Benton County, Indiana; and six pheasants shot near Brookings, South Dakota. All of the above collections were made in the spring of 1971.

RESULTS AND DISCUSSION

Effects on Reproduction

The averages of eggs laid per hen per day among hen treatment groups were significantly different in 1970 (Table 1). The rate of egg production among hens given 12.5 mg PCB was not found to be lower ($P > 0.05$) than the rate of the control group; but the rate of the 50-mg group was lower ($P < 0.01$) than the rates of the other groups. In 1971, the same pattern was evident (Table 1). There were significant differences ($P < 0.05$) among hen groups. The production of hens given 12.5 mg was not lower ($P > 0.05$) than that of controls, while production of 50-mg hens was lower ($P < 0.01$). Egg production was lower in groups where cocks were given PCB for some unexplainable reason. M. A. Haegele at the Denver Wildlife Research Center (personal communication) found that a single oral dose of 500 mg/kg body weight of Aroclor 1254 given to Japanese quail reduced egg production about 50 percent for 7 days post-treatment, but that egg production was near normal 8 to 9 days after treatment.

Differences among groups in egg fertility in 1970 were significant ($P < 0.05$); however, a significant interaction between cocks and hens ($P < 0.01$) indicated these results to be of no apparent biological meaning (Table 1). In 1971, there were no significant differences in fertility among groups.

Hatchability in both 1970 and 1971 was highest in control groups and lowest in groups in which hens had received 50 mg PCB. In

Table 1. Reproductive statistics from control pheasants and pheasants given PCB, 1970-71.

Treatment Group[a]	No. Eggs Per Hen Per Day	No. Eggs Set in Incubator	Fertile Eggs		Fertile Eggs Hatched		Fertile Eggs Pipped But Not Hatched	
			No.	Percent	No.	Percent	No.	Percent
			1970					
0-0[b]	0.621	277	101	36	74	73	6	6
0-12.5	0.459	220	128	58	87	68	29	23
0-50	0.292	136	50	37	32	64	8	16
25-0	0.335	168	91	54	73	80	9	10
25-12.5	0.362	179	71	40	50	70	18	25
25-50	0.198	98	63	64	41	65	10	16
			1971					
0-0	0.337	168	82	49	65	79	6	7
0-12.5	0.436	199	128	64	64	50	20	16
0-50	0.328	148	96	65	51	53	4	4
25-0	0.465	233	170	73	116	68	8	5
25-12.5	0.385	288	153	53	109	71	15	10
25-50	0.228	95	42	44	10	24	5	12

[a] Each treatment group had five hens, except that the 25-12.5 mg group had nine hens in 1971.

[b] The first number is the weekly PCB level in mg given to cocks, the second that for hens.

1970, these differences tested by chi-square were not significant ($P>0.05$), while in 1971 hatchability was reduced ($P<0.01$) among hens given PCB. Significant differences ($P<0.05$) were found in the number of eggs that were pipped but not hatched for hen groups in both 1970 and 1971. Apparently, the administration of PCB to the hen adversely affected the viability of the embryo at hatching. McLaughlin et al. (1963) injected both 10 and 25 mg of Aroclor 1242 into chicken eggs and found only 0 to 5 percent hatchability, growth retardation, edema, and beak deformities in embryos.

Eggshell thickness was measured only in 1970, and no significant differences ($P>0.05$) were found using analysis of variance (Table 2). Eggshells from hatched eggs (without membranes) laid by control hens averaged 0.26 ± 0.02 mm standard deviation, and those from all hen groups receiving PCB averaged 0.23 ± 0.02 mm. Eggshells from unhatched eggs averaged 0.32 ± 0.02 mm for control hens and 0.31 ± 0.02 mm for hens receiving PCB. Dahlgren and Linder (1970) found the eggshell thickness of pheasants to be unaffected by weekly administration of capsules containing 6 mg dieldrin.

Heath et al. (1970) fed Aroclor 1254 in the feed at 25 ppm to mallards through two breeding seasons. They also fed 50 ppm PCB and at a joint level of 25 ppm PCB and 15 ppm DDE to bobwhite quail for one reproductive season. They found no effects in either species on such reproductive parameters as egg production, cracked eggs, eggshell thickness, embryonation, embryos alive at 3 weeks, normality of hatchlings, and normal hatchlings alive at 14 days.

Table 2. Thickness of eggshells from eggs laid by control pheasants and pheasants given PCB, 1970.

Treatment Group	Weekly Egg Groups 1-7		Weekly Egg Groups 8-15			
	No. Hatched Eggs	Average Thickness Without Membrane (mm)	No. Hatched Eggs	Average Thickness Without Membrane (mm)	No. Unhatched Eggs	Average Thickness With Membrane[b] (mm)
0-0[a]	41	0.25	30	0.24	63	0.32
0-12.5	42	0.24	45	0.23	28	0.31
0-50	19	0.24	12	0.24	37	0.31
25-0	30	0.27	41	0.27	27	0.33
25-12.5	17	0.23	30	0.23	28	0.31
25-50	35	0.23	5	0.24	13	0.30

[a] The first number is the weekly PCB level in mg given to cocks, the second that for hens.

[b] Unhatched eggs were measured only from weekly egg groups 8-15.

Scott et al. (1971) fed 0, 0.5, 1.0, 10.0, and 20.0 ppm Aroclor 1248 for 8 weeks to chickens in full egg production and found no reduction in egg production on the lowest levels of PCB after 8 weeks. However, they noted a 10 percent reduction was associated with 10 ppm and a 13 percent reduction was associated with 20 ppm. They reported that 10 ppm Aroclor 1248 reduced hatchability of chicken eggs by 8 percent after 4 weeks and 44 percent after 8 weeks. Eggs from hens fed 20 ppm had nearly zero hatchability. Most embryos died at 21 days of development, many after pipping. They also found that eggshell strength was not affected when 20 ppm was fed.

Effects on Behavior

Birds given one 210-mg capsule in the evening of the first day of testing appeared weak at the end of the following day. After receiving the second capsule, they sat with feathers fluffed and occasionally tremored. During the 24 hours before death, they often tremored, particularly when disturbed. Shortly preceding death, birds were comatose and died without tremors.

Birds given 20 mg daily were hyperexcitable after 4 days of PCB treatment. After 10 days they sat with feathers fluffed. They exhibited weakness with occasional tremors about 30 days after dosage began. Birds on 10 and 20 mg appeared to have fewer body feathers after 30 days than controls, but they did not appear to peck one another more often. Flick et al. (1965) reported feather loss in chicks of the domestic chicken given PCB.

Visual Cliff Behavior.-- In 1970, when chicks were placed on a visual cliff for up to 5 minutes, significant differences ($P<0.01$) among groups were found in their behavior using chi-square analysis (Table 3). Among offspring of hens on 50 mg PCB weekly, 7 jumped to the visually deep side, 11 jumped to the shallow side, and 11 made no choice in the allotted time. This was in sharp contrast to the behavior of all other groups combined, wherein 15 jumped to the deep side, 132 to the shallow side, and 39 made no choice. In 1971, no significant differences in behavior between groups were noted (Table 3). When data from both years were combined, no significant differences ($P>0.05$) were found. Baxter et al. (1969) reported that pheasant chick behavior in a visual-cliff test was apparently affected by dieldrin given their parents.

Response to Hand Catching.-- Results of catching young pheasants by hand were analyzed by comparing the number of birds from each treatment group caught in the first half and last half of all birds caught to an expected number equaling 50 percent of the birds in that group. For example, of 20 young in the category where both parents had received PCB, 16 were caught in the first half of all birds caught on July 22, 1970 (Table 4). The 16 caught in the first half and 4 caught in the second half were compared by chi-square analysis to an expected 10 (half the total category of 20), resulting in a highly significant difference ($P<0.01$). Since each pen was a closed unit and the PCB-treatment birds were penned with other young from a concurrent

Table 3. Behavior on the visual cliff of chicks hatched from control pheasants and pheasants given PBC, 1970-71.

Treatment Group	No. That Jumped to Visually Deep Side		No. That Jumped to Visually Shallow Side		No. Not Jumping Within 5 Minutes	
	1970	1971	1970	1971	1970	1971
0-0[a]	2	9	35	43	8	7
0-12.5	3	7	42	34	8	5
0-50	3	7	6	30	5	6
25-0	5	16	33	67	15	17
25-12.5	5	16	22	78	7	7
25-50	4	0	5	9	6	1

[a] The first number is the weekly PCB level in mg given to cocks, the second that for hens.

Table 4. Effects of PCB on hand capture of penned pheasants, 1970-71. (Numbers represent birds caught in the first half of all birds caught; numbers in parentheses represent one-half the number of that category in the pen. Chi-square was used to compare numbers actually caught with half of the numbers in each category.)

Dates of Capture	No. of Hatches Caught	No. of Pens	Parents Receiving PCB			
			Both	Hens Only	Cocks Only	Neither
			1970			
July 22	1-8	14	16(10)**	8(8.5)	7(10.5)	11[a](10.5)
July 29	1-9	15	19(11.5)**	7(10)	9(12)	6(13.5)**
Aug. 13	1-10	18	11(12.5)	12(12.5)	12(13.5)	8(13)*
Aug. 19	1-12	20	21(15.5)*	17(14)	18(17.5)	19(18)
Sept. 1	1-14	22	25(18)*	19(15.5)	22(20)	14(19.5)
Oct. 15	1-15	23	20(16)	14(16)	25(17.5)*	9(14)
Nov. 21	1-15	13	14(11.5)	14(12.5)	8(10.5)	12(11.5)
Dec. 5	1-15	13	11(11)	14(11.5)	12(10.5)	6(10)**
All 1970 Catches Combined			137(106)**	105(100.5)	113(112)	85(110)**
Ratio, 1970[b]			1.55	1.91	1.98	2.59

Table 4 Continued

Dates of Capture	No. of Hatches Caught	No. of Pens	Both	Hens Only	Cocks Only	Neither
			1971			
July 21	1-7	17	27 (19.5)*	23(24.5)	25(21.5)	12(14.5)
Sept. 1-3	1-15	28	75(70.5)	41(37)	39(36.5)	21(22)
Sept. 21-23	1-15	28	71(70.5)	38(36)	37(36)	24(21.5)
Oct. 7-11	1-15	28	33(37)	33(35.5)	27(35)	19(20.5)
Oct. 19-22	1-15	28	34(35.5)	23(33.5)*	35(34.5)	17(21)
Nov. 2-5	1-15	28	30(30.5)	29(32.5)	23(28.5)	19(18)
All 1971 Catches Combined			270(264.5)	187(199)	187(192)	112(117.5)
Ratio, 1971			1.96	2.13	2.05	2.10
All 1970-71 Catches			407(370.5)**	292(299.5)	300(304)	197(227.5)**
Ratio, 1970-71			1.82	2.05	2.03	2.31

[a] Totals of row numbers may exceed one-half total birds because odd numbers of birds in pens were rounded higher.

[b] Birds in pen/birds caught in first half.

* $P<0.05$.

** $P<0.01$.

dieldrin study, it must be remembered that birds of several groups caught predominantly in the first half would cause birds of other groups to be caught in the last half. It is important to compare not only how the PCB birds were caught in comparison to an expected distribution but also how categories compared with one another. The comparisons are shown in Table 4 as ratios. The ability of penned pheasants to avoid hand capture was significantly less in 1970 offspring where both parents had been given PCB. This is identical to the findings of Dahlgren et al. (1970) for offspring of parents administered dieldrin. However, in 1971, the response of offspring of treatment groups was similar to controls.

Data for 1970 and 1971 were combined and a contingency table chi-square technique was used to determine the effect of PCB treatment on the response to hand catching between groups. The response was similar whether PCB were given to either the cock or hen but was significantly different ($P < 0.05$) when both parents received PCB from that where only one parent received PCB.

Effects on Survival and Weights of Offspring

Chick survival during the first 6 weeks of age while chicks were kept in brooders was determined for treatment groups in 1970 and 1971 (Table 5). The survival of chicks was not related to whether cock parents received PCB, but chi-square analysis showed that significantly more ($P < 0.01$) deaths occurred among chicks hatched from hens receiving 50 mg PCB in 1970. These differences were not evident in 1971 data.

Table 5. Weights and survival for the first 6 weeks of offspring from control pheasants and pheasants given PCB, 1970-71.

Treatment Group	Average Weight at 6 Weeks (g)		No. of Chicks to Brooder		No. of Chicks Alive After 6 Weeks		Percent Survival		
	1970	1971	1970	1971	1970	1971	1970	1971	Both Years
0-0[a]	396	429	73	65	49	50	67	77	72
0-12.5	378	435	84	64	49	45	58	70	64
0-50	303	425	29	50	3	37	10	74	51
25-0	389	428	72	115	50	91	69	79	75
25-12.5	403	458	50	109	30	72	60	66	64
25-50	344	373	40	10	9	6	22	60	30

[a] The first number is the weekly PCB level in mg given to cocks, the second that for hens.

When data from both years were combined, there was a significant difference ($P<0.01$) between offspring where hens received 0 and 12.5 mg PCB and between offspring where hens received 12.5 mg and 50 mg PCB ($P<0.01$).

Survival of young pheasants in outdoor pens was measured in December, 1970, and November, 1971 (Table 6). Survival of offspring from 6 weeks of age to the fall in both years appeared to be unaffected by level of treatment in either year. When overall survival from hatching to the fall in both years was considered, the overall survival of offspring from hens given 50 mg was significantly less ($P<0.05$) than that of the other groups. The poorer rate of survival for offspring of the hens given 50 mg was due to the effect on early survival.

When sexes of offspring in the fall were determined, no meaningful departures from the expected 50:50 sex ratios in the treatment groups were found (Table 7). Apparently, PCB did not affect the survival of one sex more than another.

Weights of chicks from hens on 50 mg PCB weekly were lower ($P<0.01$) at 6 weeks of age than those of other groups in 1970 (Table 5). In 1971, weights of offspring of 50-mg hens were lower ($P<0.01$) than that of controls, while weights of offspring of 12.5-mg hens were higher ($P<0.01$). When data from both years were combined, there was no relationship between weight and treatment levels.

Although it was not adequately determined that PCB via the egg depressed the weight of offspring, McLaughlin et al. (1963) mentioned

Table 6. Survival to the fall of offspring of control pheasants and pheasants given PCB, 1970-71.

Treatment Group	No. of Young Alive in Fall[a]		Percent Survival From 6 Weeks of Age to Fall[b]			Percent Survival Hatching to Fall[c]		
	1970	1971	1970	1971	Both Years	1970	1971	Both Years
0-0[d]	20	36	41	72	57	27	55	41
0-12.5	23	36	47	80	63	27	56	40
0-50	0	29	0	78	72	0	58	37
25-0	21	57	42	63	55	29	50	42
25-12.5	18	59	60	82	75	36	54	48
25-50	4	4	44	67	53	10	40	16

[a] December 5, 1970; November 2-5, 1971.

[b] Using the number of chicks alive after 6 weeks, Table 5.

[c] Using the number of chicks to brooder, Table 5.

[d] The first number is the weekly PCB level in mg given to cocks, the second that for hens.

Table 7. Numbers of cocks and hens alive in November that were offspring of control pheasants and pheasants given PCB, 1970-71. (Chi-square was used to compare numbers of each sex alive with an expected 50:50 distribution.)

Treatment Group	1970 November 21		1971 November 2-5		1970-71 Combined	
	Cocks	Hens	Cocks	Hens	Cocks	Hens
0-0[a]	11	11	17	17	28	28
0-12.5	14	11	15	20	29	31
0-50	0	1	13	17	13	18
25-0	5	16*	33	22	38	38
25-12.5	13	6	36	20*	49	26**
25-50	1	1	3	1	4	2
All PCB Groups	33	35	100	80	122	115

[a] The first number is the weekly level in mg of PCB given to cocks, the second that for hens.

* $P < 0.05$.

** $P < 0.01$.

growth retardation of embryos as an effect of PCB injected into the yolk sac of chicken eggs. Flick et al. (1965) found that 1-day-old chickens fed Aroclor 1242 at 200 and 400 ppm had depressed growth by the second week of feeding and that the growth depression was related to the level fed. Vos and Koeman (1970) fed PCB to 1-day-old cockerels and found body-weight depression from 400 ppm Aroclor 1260. Platonow and Funnell (1971) found that 1-day-old cockerels fed 250 ppm Aroclor 1254 had depressed body weights between the sixth and ninth week of their feeding trial. This depression was associated with reduced feed consumption. Rehfeld (1971) also found depressed weight gains in 1-day-old chicks fed sublethal levels (10-50 ppm) of Aroclor 1254. Chicks fed 30 and 50 ppm for 2.5 weeks and then fed a clean diet recovered from the growth depression, while chicks fed 40 and 50 ppm for 5 weeks and then fed a clean diet for 8 weeks did not show a recovery from the growth depression.

Body Weights and Mortality in Birds Given PCB

Adult hens in both the 1970 and 1971 breeding experiments were unaffected in body weight by administration of as much as 50 mg PCB in single capsules weekly. This was determined by analysis of variance comparing weekly changes from initial weight. It was characteristic, however, of birds that died of PCB toxicosis to stop eating and die within several days.

Hens 11-weeks old given a 210-mg capsule of PCB in the evening ate very little the following day and appeared weak by the end of that

day. After receiving the second capsule 24 hours subsequent to the first, birds sat with feathers fluffed, tremored occasionally, and did not eat. Birds of both sexes 6 to 9 months of age given 100 mg or 50 mg PCB every 3.5 days for 5 weeks continued to eat, as did 11-week-old hens repeatedly given either 10 or 20 mg daily. Scott et al. (1971) found that up to 20 ppm Aroclor 1248 fed to laying chickens had no effect on feed consumption and no mortality could be attributed to treatment. Prestt et al. (1970) found no effect on weight of Bengalese finches when they were fed up to 400 ppm Aroclor 1254 for 56 days.

Birds 11-weeks old that were given capsules daily containing 210 mg PCB died within 1.3 and 5.9 days. The 16 designated to be analyzed upon death lived longer than the other birds in the experiment, from 2.2 to 5.9 days, averaging 3.8 ± 1.0 day standard deviation. The latter lost from 15 to 37 percent of their initial weight before death. All control birds lived. Correlations of the initial weight, days to death, and percentage weight loss were calculated for 53 birds in this experiment. Initial weight was correlated with days to death ($r = 0.699$, $P<0.01$). The heaviest birds lived longest. The birds which were heaviest initially lost the greatest percentage of their weight before death ($r = 0.589$, $P<0.01$). Birds that lived the longest lost the greatest percentage of their weight before death ($r = 0.744$, $P<0.01$). Time of death of the 11 birds that were not given PCB but were starved ranged from

2.3 days to 8.6 days, averaging 3.9 ± 1.8 days. They lost from 27 to 51 percent of their weight by the time of death.

Of the birds given 50 or 100 mg every 3.5 days for 5 weeks, 4 of 22 died in the 50-mg group and 7 of 22 died in the 100-mg group. In addition, two birds in the 100-mg group were so weak they were near death at the conclusion of the experiment.

Mortality in birds given 10 mg daily began 30.6 days after the first capsule was given. The ninth bird died after 179.3 days. The other seven birds died between 50.3 and 60.6 days after initial treatment. The tenth bird of this group, still alive after 8 months of treatment, was sacrificed.

In the 20-mg group, the first bird died 39.6 days and the last bird 54.1 days after capsules were first given. The average number of days to death was 46.1 ± 5.3 days.

Mortality was low in breeding experiments in 1970 and 1971. In 1970, only two hens died from among the 30 hens and 10 cocks under study. Both hens had received 50 mg PCB weekly. In 1971, of the 34 hens and 14 cocks in the study, three hens died, two that had received 12.5 mg and one that had received 50 mg weekly.

Tucker and Crabtree (1970) reported that 2000 mg/kg body weight of either Aroclor 1242, 1254, 1260, or 1268 given to mallards caused no mortality or symptoms. Prestt et al. (1970) estimated that 254 mg/kg/day given to Bengalese finches would give 50 percent mortality at 56 days. Heath et al. (1970) reported that Aroclor 1254 had an LC_{50} of 1090 ppm when fed for 5 days as part of the diet to pheasants.

Vos and Koeman (1970) reported that of 20 chicks fed 400 ppm for 60 days only 3 died.

In the present study, 210 mg PCB daily were given to 11-week-old hens. A bird dying at the average number of days to death would have received 840 mg PCB, the first bird of the group to die received 420 mg, and the last 1260 mg. These same figures for 11-week-old hens on 20 mg daily were 940 mg, 800 to 1100 mg, and for birds given 10 mg daily (one was sacrificed after surviving 8 months) were 830+ mg, 310 to 2410+ mg. Thus, for a group of pheasants given PCB at 10 mg or more daily, the most susceptible individuals would probably die with 300 to 400 mg Aroclor 1254, the average bird would die with a cumulative dose totaling from 800 to 950 mg, and the least vulnerable would die after receiving 1200 to 2410+ mg PCB.

Absorption, Storage, and Excretion

To determine the absorption and storage patterns of PCB in pheasants, tissues from the brain, liver, muscle, and whole body were analyzed for residues from (1) groups of four hens given a single 50-mg capsule and sacrificed 12 hours, 24 hours, 7 days, and 28 days later; (2) hens given 12.5 mg or 50 mg weekly for 17 weeks during the 1970 breeding experiment; and (3) hens given 12.5 mg weekly for 16 weeks in the 1971 breeding experiment that were killed 1 week after the last capsule and 3 and 6 months later.

The average percentage recovery and standard deviation of Aroclor 1254 from tissue of pheasant hens injected with 2, 20, and

200 ppm based on live weight was 94 ± 9, 99 ± 6, and 91 ± 12 percent, respectively. When 2, 20, and 200 ppm of PCB were added to ground whole-body control samples, the average percentage recovery was 84 ± 6, 94 ± 5, and 103 ± 6, respectively. Therefore, the average percentage recovery of PCB from metabolically-incorporated or fortified samples was 94 ± 7 percent.

Retention of PCB in the bodies of four hens each given single 50-mg capsules and sacrificed 28 days later was calculated from the data for whole body analysis and weight at death (Table 8). The retention averaged 40.5 mg in each hen. Prestt et al. (1970) fed 1500 mg Aroclor 1254 over 56 days to Bengalese finches and were able to recover only 9 percent of the amount fed when they analyzed one bird from the experiment.

Whole bodies of control hens checked at the beginning of the experiment where single 50-mg capsules were given to hens had 0.6 ppm PCB (Table 8). There was less than 0.5 ppm in brain, liver, and muscle tissues of these birds (Table 9). Levels of PCB were found to be highest in all tissues at 12 hours after capsule administration, declining most rapidly in liver at 24 hours (Table 9). Throughout the experiment, levels were highest in liver followed by brain and muscle. This pattern is similar to that found by Lamb et al. (1970) for pheasants given dieldrin.

Brain tissue contained more PCB than muscle per unit weight but, having a higher lipid percentage than muscle tissue, had a smaller concentration of PCB per unit of lipid.

Table 8. Whole-body analyses of four pooled control hens and a pooled sample of four hens killed 28 days after each was given a single capsule containing 50 mg PCB, 1970.

Sample	Total Dose Per Bird	Average Death Weight (grams)	Percentage Dry Matter	Percentage of Lipid (Ether Extract)	Wet Weight PCB (ppm)	Total PCB in Body (mg)
Control	0 mg	1276	40.8	9.1	0.6	0.8
Treated	50 mg	1286	41.4	12.4	30.6	40.5

Table 9. Levels of PCB found in brain, liver, and muscle at time intervals after administration of a single capsule containing 50 mg PCB, 1970. Samples from four hens were pooled.

Tissue and Time Interval	Percentage of Dry Matter	Percentage of Lipid (Ether Extract)	Wet Weight PCB (ppm)
	Brain		
0 hours	22.5	3.5	<0.5
12 hours	22.2	3.9	19.4
24 hours	23.4	3.1	8.0
7 days	23.1	3.4	2.8
28 days	22.0	3.1	2.5
	Liver		
0 hours	30.6	5.5	<0.5
12 hours	29.8	2.1	71.8
24 hours	29.8	2.8	25.7
7 days	29.9	2.4	16.2
28 days	32.8	3.0	19.1
	Muscle		
0 hours	29.1	0.5	<0.5
12 hours	29.2	0.6	10.8
24 hours	29.2	0.7	5.6
7 days	29.6	0.5	2.3
28 days	29.6	0.7	1.9

Analysis of whole bodies of four hens that received 12.5 mg PCB weekly for 17 weeks revealed that they retained from 37 to 56 percent of the administered dose, while hens on the 50-mg level retained 60 to 82 percent of the administered dose (Table 10). The hens on the higher dose averaged about six times as much PCB in their bodies as the hens on the lower dose, although they had been given only four times as much PCB.

A total of 48.7 mg of PCB in the whole body plus that excreted in eggs and feces over 28 days was accounted for after administration of a 50-mg capsule. The PCB-corn oil mixture was readily absorbed in the gut of the pheasant. Calculations showed that a maximum amount excreted unabsorbed was 6 percent of the 50 mg given and as much as 98 percent may have been absorbed.

Five of the hens that had received 12.5 mg weekly for 16 weeks in the 1971 breeding experiment were sacrificed 1 week following the administration of the last capsule in the series. The ppm PCB in the bodies of these five hens were 17.6, 18.6, 24.4, 28.3, and 30.5, averaging 23.8 ppm. Three months later, three other hens of the same group which had been kept caged were sacrificed. The analyses of their bodies showed 8.9, 12.0, and 20.0 ppm PCB, averaging 13.6 ppm PCB. After 6 months on a clean diet, three other birds similarly treated had whole-body ppm values of 18.3, 19.5, and 25.0, averaging 20.9 ppm. The apparent rise in PCB concentration at 6 months may be due to sample variation and/or the physiological state of the hens sampled.

Table 10. Whole-body analyses of pheasant hens given single capsules of 12.5 and 50 mg of PCB weekly for 17 weeks and sacrificed 1 week later, 1970.

Bird Number	Level of Dose (mg)	Death Weight (grams)	Percentage of Dry Matter	Percentage of Lipid (Ether Extract)	Wet Weight PCB (ppm)	Total PCB in Body (mg)	Total Dosage Given (mg)	Percentage of PCB Retained
210	12.5	1,325	40.9	10.2	61	81	212.5	38
218	12.5	1,309	39.0	10.8	60	79	212.5	37
219	12.5	1,421	44.6	16.1	84	120	212.5	56
242	12.5	1,020	35.8	5.0	116	118	212.5	56
223	50.0	1,188	43.1	17.0	516	613	850.0	72
233	50.0	1,405	40.6	11.2	456	641	850.0	75
234	50.0	1,404	44.1	14.8	497	698	850.0	82
237	50.0	1,096	38.1	9.8	468	513	850.0	60

It is obvious that the rate of excretion of PCB is relatively slow. These birds were analyzed at the Denver Wildlife Research Center.

Scott et al. (1971) found that chicken hens given up to 20 ppm Aroclor 1248 in the diet for 8 weeks lost less than 50 percent of the stored PCB after 4 weeks on either a standard diet or low-energy diet. They also found that a low-energy diet followed by a high-energy diet did not affect reduction of PCB over time. Prestt et al. (1970) fed 1500 mg Aroclor 1254 over 56 days to Bengalese finches and were able to recover only 9 percent of the amount fed when they analyzed one bird from the experiment.

Excretion Via the Feces.-- Excretion of PCB in feces was relatively low. An average of 4.0 mg per single 50-mg dose was excreted in the feces of each of four hens over 28 days. Two other hens excreted 2.2 and 2.9 mg per 50-mg dose. PCB in the feces of the four hens were at a peak during the first week and declined to relatively lower levels thereafter (Table 11). Excretion in the feces was highest during the first 24 hours in two other hens given single 50-mg capsules (Table 12), and less than 1 mg PCB appeared in the feces the first day. Variability in excretion between these two hens was low. An average of 2.6 mg per hen was passed in the feces of the two hens by the end of 28 days.

Excretion Via the Egg.-- Egg laying was declining when the 50-mg capsules were given to the four hens. These hens laid a total of four eggs the first week, two the second, five the third, and five the

Table 11. Levels of PCB found in whole eggs and feces after administration of a single capsule containing 50 mg of PCB, 1970. Samples from four hens were pooled.

Time Interval (days)	Eggs						Feces		
	Total Number Laid	Number Analyzed	Average Weight (grams)	Percentage of Dry Matter	Percentage of Lipid (Ether Extract)	Wet Weight PCB (ppm)	Total Dry Weight	Percentage of Lipid (Ether Extract)	Dry Weight PCB (ppm)[a]
0-7	4	4	32.0	33.1	10.5	25.3	494	0.6	24.9
8-14	2	1	30.5	29.4	7.9	49.1	479	0.6	3.1
15-21	5	2	33.5	38.9	8.4	34.0	466	0.8	2.8
22-28	5	4	33.3	40.7	9.1	26.8	471	0.5	2.2

[a] Average of a sample and duplicate from same pool.

Table 12. Levels of PCB found in feces of two nonlaying pheasant hens after administration of a single capsule containing 50 mg of PCB, 1970.

Time Interval (days)	Bird Number	Dry Weight (grams)	Dry Weight PCB (ppm)	PCB (mg)
0-1	I	27.6	24.3	0.67
	II	40.6	24.2	0.98
2-7	I	231.6	2.3	0.53
	II	243.9	2.9	0.71
8-14	I	306.9	1.5	0.46
	II	308.0	1.8	0.55
15-21	I	237.6	0.9	0.21
	II	292.5	1.0	0.29
22-28	I	230.4	1.1	0.25
	II	291.2	1.1	0.32

fourth week. Levels of PCB in the eggs were lowest in the first week (one egg was laid the first day, one the second, and two the fourth), highest during the second week, and declined thereafter (Table 11). The average excretion per hen via egg production was calculated to be 4.2 mg PCB.

Excretion of PCB via the egg could be higher than excretion through the feces when hens are in full egg production. A single egg laid between 1 and 2 weeks after a hen was given a single capsule containing 50 mg PCB was shown to contain 1.5 mg of PCB. This egg was one of two laid that week by one hen. Egg production may be an important means of excretion for the hen, but such excretion may be dangerous to the offspring in terms of increased mortality at the pipping stage and an altered behavior pattern.

Heath et al. (1970) reported that two eggs laid in the second reproductive season by mallards fed 25 ppm Aroclor 1254 had 56 ppm and 33 ppm PCB, wet weight. These results are comparable to those we found at time intervals following a single 50-mg dose (Table 9). Peakall (1971) reported that ring dove eggs taken from birds given 10 ppm Aroclor 1254 in the diet averaged 4.81 $\pm$ 1.08 ppm standard error. The actual amounts of PCB in the ring dove and mallard eggs and the pheasant eggs in the present study would be far below the 10 mg injected by McLaughlin et al. (1963) into chicken eggs. The latter resulted in poor hatchability, edema, and beak deformation.

Scott et al. (1971) found that PCB deposited in chicken eggs were less than 0.5 ppm after 8 weeks with 0.5 and 1.0 ppm Aroclor 1248

in the diet. They found levels of over 3 ppm after 8 weeks with 10 ppm and levels of about 6 to 7 ppm in eggs of hens on 20 ppm. These values are much lower than those found in the present study from 3 to 4 weeks after administration of a single capsule of 50 mg Aroclor 1254. Results of Scott et al. (1971), showing a drastic reduction in hatchability associated with 7 ppm or less residue in eggs, are not comparable to findings in this study with pheasants. Differences with the experimental animal or the Aroclor product used may have resulted in the gross differences in findings between the studies. Heath et al. (1970) reported a nearly fourfold difference between bobwhite and Japanese quail in the LC_{50}, thus species differences may be important.

Residues in eggs of wild birds have been determined by several authors from diverse collection points. Anderson et al. (1969) found PCB in all egg pools of cormorants (Phalacrocorax auritus) and pelicans (Pelecanus erythrorhynchos). Cormorants were shown to have an estimated 8 ppm in eggs and pelicans 0.6 ppm. Jensen et al. (1969) reported 48 ppm in the egg of a heron (Ardea cinerea) collected in Sweden and 8 to 21 ppm from 9 guillemot (Uria aalge) eggs from the Baltic Sea. Risebrough et al. (1968a) reported that an egg of a peregrine falcon (Falco peregrinus) contained 10 ppm, 8 black petrel (Loomelania melania) eggs had an average of 1 ppm, 5 eggs of a barn owl (Tyto alba) had <1 ppm, and a golden eagle egg (Aquila chrysaetos) had <1 ppm. Dustman et al. (1971) reported median measurements of egg residues to be 1.65 ppm for Alaskan bald eagles and 9.7 ppm PCB for those from the United States. They further

reported median egg levels of 15.9 ppm for the osprey (Pandion haliaetus) of Connecticut and 2.5 ppm for those in Maryland, 5 to 6 ppm in brown pelican (P. occidentalis) eggs from different areas, and 5 ppm in eggs of royal terns (Thalasseus maximus).

Prestt et al. (1970) found residues ranging from 0 to 80 ppm in 363 eggs from 28 species of both land and water birds collected in Britain. Most of these had less than 10 ppm, but those which had higher levels included a single egg of the great crested grebe (Podiceps cristatus), 40 ppm; one egg among 101 of the heron that had 80 ppm, while the arithmetic mean was 5 ppm; one egg of a moorhen (Gallinula chloropus) with 15 ppm among 13 samples that averaged 2.4 ppm; and a single egg of a great skua (Stercorarius skua) that had 25 ppm.

Particularly in view of findings by Scott et al. (1971) that about 5 ppm were associated with nearly complete negation of hatchability in the chicken, the above results for some wild birds are alarming. Apparently, though, as earlier pointed out, there must be considerable differences among species in (1) the rate at which they deposit PCB in the egg and (2) what a particular ppm range may mean in associated deleterious effects.

Residue Levels in Tissues

Tissues were analyzed from seven different groups of birds: (1) 16 birds given 210 mg PCB daily that were designated for analysis upon death, (2) five additional birds on 210 mg daily that died,

(3) nine birds that were killed at intervals for matching with the 16 designated to die, (4) pooled samples of birds killed 12 hours and 24 hours after receiving a single capsule containing 210 mg PCB, (5) pooled samples of four birds dying during treatment and four birds surviving capsules containing 50 and 100 mg PCB, (6) a pooled sample of four birds dying on 20 mg and 10 mg PCB, and (7) a pooled sample of two control birds (Table 13).

Birds that died from daily doses of 210 mg PCB had brain levels that ranged from 320 to 770 ppm wet weight and averaged 520 ppm ± 110 ppm standard deviation. Liver residue levels were much more variable than brain levels, ranging from 390 to 9,300 ppm and averaging 2,500 ± 2,000 ppm wet weight. Muscle residues were also relatively more variable than brain residues, as they ranged from 51 to 290 ppm and averaged 140 ± 53 ppm. Tissue levels from apparently healthy birds that were sacrificed overlapped with ranges in the birds that died on 210 mg but less so for brain than for liver or muscle ranges. Brain residue levels of nine birds sacrificed for matching of brain residue levels ranged from 280 to 500 ppm and averaged 370 ± 65 ppm; liver residues ranged from 1,000 to 5,000 ppm and averaged 1,900 ± 1,300 ppm; and muscle residues ranged from 58 to 110 ppm and averaged 83 ± 17 ppm.

Brain residues were less variable than residues in other tissues. Further, brain was the only tissue that had residues that were independent of other parameters as determined by testing correlations of all possible parameters, using birds that died from

Table 13. Results of residue analyses of PCB in brain, liver, and muscle tissues from birds that died of PCB toxicity and from surviving treated birds that were sacrificed.

Treatment	Bird Number	Initial Weight	Death Weight	Days to Death	Brain			Liver			Muscle		
					Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)	Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)	Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)
Died 210 mg[a]	61	727	502	3.5	7.04	7,100	500	2.59	33,000	860	1.43	10,000	150
	66	772	539	4.3	7.33	11,000	770	3.57	62,000	2,200	1.14	13,000	150
	77	761	548	4.4	5.52	11,000	590	5.48	80,000	4,400	0.40	72,000	290
	333	678	504	3.4	5.00	8,400	420	--	--	--	0.81	11,000	93
	335	761	628	2.8	6.97	7,700	540	4.17	53,000	2,200	0.32	50,000	160
	347	693	553	3.0	7.67	6,600	510	4.87	70,000	3,400	0.29	74,000	210
	351	823	551	4.7	7.10	8,500	600	5.98	47,000	2,800	0.42	26,000	110
	356	653	525	2.5	5.88	7,500	440	2.81	14,000	390	0.34	15,000	51
	361	715	521	3.4	6.30	9,800	620	5.45	35,000	3,800	0.43	35,000	150
	366	770	530	4.4	5.52	12,000	670	4.18	62,000	2,600	0.23	66,000	150
	367	625	527	2.2	4.83	9,700	470	2.36	36,000	860	0.58	16,000	90
	375	839	526	5.9	6.55	7,600	500	10.94	85,000	9,300	0.32	62,000	200
	381	760	576	3.9	6.15	8,600	530	3.36	63,000	2,100	0.49	28,000	140
	387	845	624	4.4	7.50	9,100	680	5.60	55,000	3,100	0.19	85,000	160
	389	783	602	3.1	6.33	7,400	470	3.33	42,000	1,400	0.47	22,000	100
	390	724	531	4.4	6.13	9,000	470	4.39	50,000	2,200	0.22	60,000	130

Table 13 Continued

Treatment	Bird Number	Initial Weight	Death Weight	Days to Death	Brain			Liver			Muscle		
					Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)	Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)	Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)
Died 210 mg[b]	70	556	470	1.3	4.29	7,500	320	--	--	--	2.96	3,100	93
	78	497	413	1.3	3.75	10,000	390	--	--	--	0.77	11,000	82
	378	658	558	1.9	6.67	5,400	360	2.08	38,000	780	1.43	7.700	110
	393	611	528	4.1	11.00	5,500	600	3.12	38,000	1,200	0.50	20,000	100
	398	708	582	2.7	8.67	5,400	470	3.04	46,000	1,400	0.28	43,000	120
Sacrificed 210 mg[c]	63	740	589	2.4	6.88	4,100	280	4.45	29,000	1,300	0.26	27,000	68
	68	802	604	3.8	5.31	9,400	500	7.02	71,000	5,000	0.38	26,000	100
	73	616	499	2.4	6.52	6,000	420	5.07	30,000	1,500	0.36	16,000	58
	74	670	574	2.9	7.60	4,800	360	5.40	35,000	1,900	0.83	9,100	76
	76	720	540	3.8	6.43	--	410	5.71	47,000	2,700	0.62	15,000	95
	328	781	576	3.6	6.13	5,500	340	4.40	27,000	1,200	0.80	14,000	110
	332	804	643	3.6	6.25	5,300	330	4.80	29,000	1,400	0.87	8,400	73
	341	760	626	2.6	6.36	5,200	330	4.50	27,000	1,200	0.24	37,000	88
	399	669	492	3.6	6.33	5,800	370	3.90	26,000	1,000	0.65	12,000	80
	1-3[d]	--	--	0.5	--	800	52	--	6,100	170	--	8,100	28
	4-6[d]	--	--	1.0	--	430	90	--	12,000	420	--	12,000	22

Table 13 Continued

Treatment	Bird Number	Initial Weight	Death Weight	Days to Death	Brain Lipid (percent)	Brain PCB in Lipid (ppm)	Brain PCB (ppm)	Liver Lipid (percent)	Liver PCB in Lipid (ppm)	Liver PCB (ppm)	Muscle Lipid (percent)	Muscle PCB in Lipid (ppm)	Muscle PCB (ppm)
Died 100 mg	A[e]	--	--	--	7.50	4,300	320	4.93	26,000	1,300	--	--	--
Survived 100 mg	B[e]	--	--	--	7.16	830	59	3.00	5,300	160	--	--	--
Died 50 mg	C[f]	--	--	--	7.06	5,000	350	3.00	21,000	640	--	--	--
Survived 50 mg	D[e]	--	--	--	7.04	480	34	3.07	2,700	84	--	--	--
Died 20 mg	E[e]	--	--	--	6.95	5,500	380	4.20	33,000	1,400	--	--	--
Died 10 mg	F[e]	--	--	--	6.59	5,500	360	3.47	35,000	1,200	--	--	--
Control	G[d]	--	--	--	5.79	<0.1	<0.1	2.60	<0.1	<0.1	--	--	--

[a] Sixteen birds designated for analysis after death.

[b] Five additional birds that died and were analyzed.

[c] Nine birds killed at intervals.

[d] Two birds pooled.

[e] Four birds pooled.

[f] Three birds pooled.

capsules containing 210 mg PCB (Table 14). This lack of correlation with other parameters indicated the usefulness of the brain in assessing toxic levels of PCB. The relationship of liver residues with other parameters such as original bird weight and days to death tended to negate its usefulness (Table 14). Further, the inter-relationships of liver parameters with those of muscle (Table 14), even though it is not known how many of these relationships were meaningful, tended to negate the usefulness of muscle as an indicator tissue.

Brain levels of PCB were generally higher in birds that died than in birds that were sacrificed at the same time (Fig. 2), although there was some overlap of the two groups. A brain residue level of 400 ppm separated the bulk of the birds that died (86 percent) from most of those that were sacrificed (67 percent). There appeared to be a relationship between days to death and brain residue level (Fig. 2); however, this was not significant ($P>0.05$). A pooled sample of four birds that died during treatment with 100 mg PCB given each 3.5 days had 320 ppm in the brain tissue, while a pooled sample of four sacrificed birds in this group had 59 ppm (Table 13). A pooled sample of three birds that died during treatment with 50 mg PCB given every 3.5 days had 350 ppm in the brain, while a pooled sample of four sacrificed from this group had 34 ppm. Pooled brain tissues from four birds that died on daily doses of 10 and 20 mg had 360 and 380 ppm PCB, respectively. No data were available for survivors of these groups. These latter data indicated that 300 ppm might be better than 400 ppm as a separation point that would indicate death from PCB

Table 14. Significant correlations between all possible parameters from 15 of 16 birds that died from daily capsules containing 210 mg PCB (Table 13, footnote a, bird number 333 excluded).

Possible Parameters	Original Weight	Percent Weight Loss	Days to Death	Brain	Liver			Muscle	
				PCB in Lipid (ppm)	Lipid (percent)	PCB in Lipid (ppm)	PCB (ppm)	Lipid (percent)	PCB in Lipid (ppm)
Original weight	--								
Percent weight loss	0.663**	--							
Days to death	0.797**	0.894**	--						
Brain									
Lipid (percent)									
PCB in lipid (ppm)				--					
PCB (ppm)				0.685**					
Liver									
Lipid (percent)	0.623*	0.609*	0.760**		--				
PCB in lipid (ppm)	0.577*		0.666**		0.625*	--			
PCB (ppm)	0.556*	0.588*	0.733**		0.966**	0.734**	--		
Muscle									
Lipid (percent)								--	
PCB in lipid (ppm)					0.532*	0.648**	0.542*	-0.664**	--
PCB (ppm)						0.813**	0.616*		0.657**

* $P < 0.05$.

** $P < 0.01$.

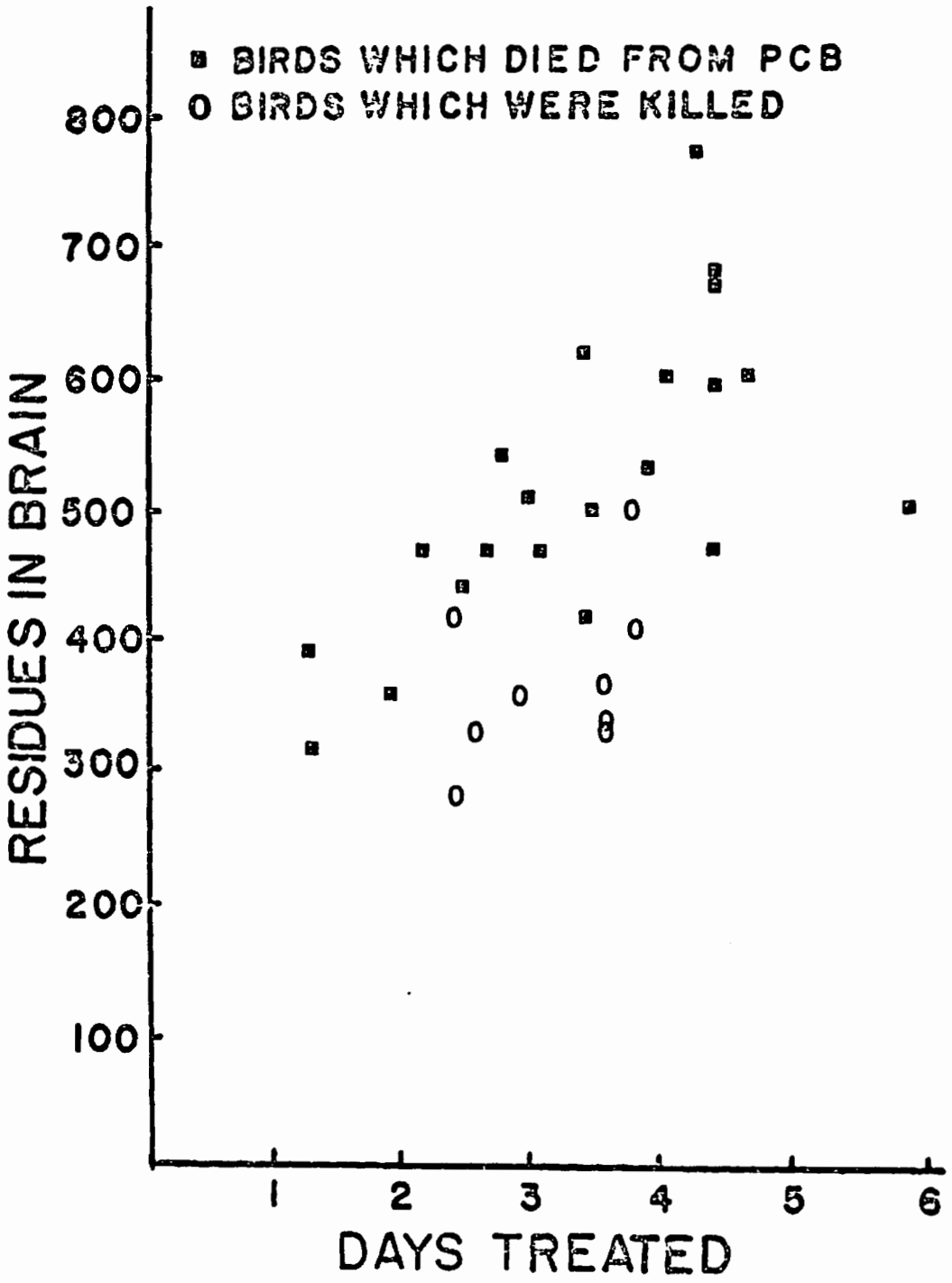


Fig. 2. The relationship of brain residue levels (ppm wet weight) between 11-week-old hen pheasants that died on 210 mg PCB daily and those on the same dosage that were killed at similar times.

and that, when smaller amounts of PCB were received by the birds over a period of time, the spread in brain levels between birds that died and survived might be greater.

Ratios of residue levels using wet-weight ppm values among brain, liver, and muscle in dead and sacrificed birds overlapped considerably, particularly in brain:liver and brain:muscle ratios. However, liver:muscle ratios appeared to be smaller in birds dying on 210-mg doses. If one establishes 19 as a liver:muscle ratio, 3 of 9 birds sacrificed on the 210-mg doses had smaller ratios and only 4 of 18 birds dying had higher ratios. However, more data must be gathered to determine if this ratio is useful in diagnosing the cause of death as PCB toxicosis.

Vos and Koeman (1970) found that, when 1-day-old cockerels were fed 400 ppm of either Phenochlor CP 6, Clophen A60, or Aroclor 1260 in the diet for 60 days, the chicks had brain residue levels ranging from 70 to 700 ppm among 11 birds that died on treatment and 40 ppm for one chick that survived. Liver levels in their experiment were more variable than brain levels and ranged from 120 to 2,900 ppm among 28 birds that died and from 210 to 340 ppm among four survivors. Seven of their 28 birds that died had liver residues of less than 250 ppm, while four of 11 birds had less than 300 ppm. It appears from their data that liver might be as useful as brain for diagnosing cause of death. Their liver:brain ratios were similar to those of the present study; but, since they had only one survivor from which brain and liver were analyzed, overlap could not be evaluated. Rehfeld (1971)

reported that 30 to 50 ppm Aroclor 1254 given to 1-day-old cockerels resulted in liver residues of 300 to 500 ppm. Prestt et al. (1970) reported a range of 3 to 634 ppm in livers of survivors and 70 to 697 ppm in livers of Bengalese finches dying during treatment with Aroclor 1254 and that liver level was correlated with the amount received ($P<0.01$).

Prestt et al. (1970) stated that the ratio of brain:liver residues should be compared and that this ratio was three times higher in Bengalese finches that died during their experiment than in birds killed at the end. Data in the present study showed a complete overlap in the brain:liver residue ratio. Birds that died during testing had a range in ratios of 0.05 to 1.13, while matching birds sacrificed had 0.10 to 0.37.

Relatively little sampling of brain tissue for PCB residues has been done in wild birds. Dustman et al. (1971) reported that a sick bald eagle had 230 ppm PCB in its brain and that PCB may have contributed to its death. Jensen et al. (1969) reported that three white-tailed eagles (Haliaeetus albicilla) had a brain residue range of 29 to 70 ppm PCB, averaging 47 ppm. Muscle residues ranged from 150 to 240 ppm, averaging 190 ppm. Risebrough et al. (1968a) found 0.04, 1.5, 21, and 34.6 ppm PCB in the brains of four peregrine falcons. Prestt et al. (1970) reported liver residues ranging from 0 to around 900 ppm from a wide variety of British birds. Arithmetic averages ranged from 0.5 ppm in the buzzard (Buteo buteo) to 98 ppm in the heron. The tissue levels reported above were below those at

which mortality occurred in the present study, except that the muscle level for the white-tailed eagle was higher than those in this study associated with death in the pheasant from PCB. However, these levels from wild birds do constitute substantial percentages of the brain and liver levels in the present study where death occurred from PCB.

Histopathologic Effects

Weights of organs taken from starved, control, and treatment birds were converted to a percentage of body weight to compensate for a variance in bird size. Analysis of variance was conducted on the converted data, and Dunnetts "t" test was used to compare starved and treated groups with controls (Table 15). PCB treatment decreased weights of heart and spleen at all levels ($P<0.01$). PCB treatment increased weights of kidney and liver in birds given 10- and 20-mg doses ($P<0.01$), but no effect was seen in the 210-mg group. Starved birds had smaller hearts ($P<0.05$) and livers ($P<0.01$) than controls.

Splenic atrophy, as described by Flick et al. (1965) and Vos and Koeman (1970), was found in all pheasants given 20 mg daily and in the 10-mg birds except for the one that survived 8 months and was sacrificed. Spleens were pale tan, small, and had wrinkled capsules. A few of the birds given 210 mg PCB daily showed a degree of splenic atrophy, but the spleens were not as small as those from birds given 10 or 20 mg and were near normal in color. Four of the 11 birds used as starvation controls also showed a degree of splenic atrophy, but the capsules were not wrinkled and the color was slightly lighter than normal.

Table 15. Weights in grams of organs from starved pheasants, controls, and those dying from daily capsules containing PCB. Weights were converted to a percentage of body weight and compared to controls using Dunnett's "t" test.

	Heart			Kidney			Liver			Spleen		
	Range	Mean	Mean Percentage of Body Weight	Range	Mean	Mean Percentage of Body Weight	Range	Mean	Mean Percentage of Body Weight	Range	Mean	Mean Percentage of Body Weight
Starvation (11)[a]	2.4-4.9	3.5	0.73*	2.8-4.3	3.6	0.75	9.0-12.3	11.1	2.29**	0.20-0.50	0.35	0.07
Controls (14)	5.0-7.8	6.2	0.87	3.4-7.8	6.0	0.83	17.2-34.3	26.5	3.71	0.20-0.80	0.61	0.09
10 mg PCB (9)	2.6-6.5	4.1	0.66**	4.2-13.1	7.5	1.18**	18.7-32.7	26.4	4.45**	0.10-0.26	0.14	0.02*
20 mg PCB (10)	2.8-4.8	3.7	0.64**	5.6-10.3	7.2	1.20**	21.5-34.6	27.4	4.59**	0.10-0.30	0.15	0.02**
210 mg PCB (34)	2.4-4.1	3.2	0.60*	3.3-6.2	4.7	0.89	12.7-22.5	18.1	3.39	0.10-0.30	0.18	0.03**

[a] Number of birds sampled.

* $P < 0.05$.

** $P < 0.01$.

Histologically, marked splenic atrophy was characterized by almost complete absence of lymphatic nodules. There was an increase in the relative abundance of red pulp. In one of the birds given 20 mg, loci of necrosis were found in lymphatic nodules. The four starved birds that exhibited a degree of splenic atrophy had some depletion of lymphatic nodules, but the lymphoid depletion was not as marked as in birds given 10 or 20 mg. Spleens of the birds given 210 mg appeared similar to those of the four starved birds mentioned above.

Livers of the birds given 10 mg and 20 mg PCB were slightly swollen and borders of the liver lobes were rounded. Liver color was pale tan. Livers of the birds starved or given 210 mg appeared smaller than normal, their borders were sharp, and color was relatively normal.

Histopathologic examination revealed some apparent early hydropic degeneration of liver cord cells in birds given 10 or 20 mg. Cytoplasm was foamy in appearance, but nuclei were normal and there was no evidence of fatty change or necrosis. Livers appeared normal in all other treatment groups.

The bird which survived for 8 months while receiving 10 mg PCB daily and was then sacrificed had a normal-appearing spleen and a pale-yellow, somewhat swollen liver but no other gross lesions. Microscopic lesions were confined to the liver. There was extensive hydropic degeneration of most of the liver cord cells in each field. Some cells located at the periphery of liver lobules had undergone fatty

change. The nuclei of these cells appeared normal but were eccentrically placed due to lipid deposits.

At necropsy, one of the birds receiving 10 mg PCB daily and one receiving 20 mg had hydropericardium. Approximately 0.4 ml of clear fluid was aspirated from each pericardial sac. None of the birds in the other treatment or control groups exhibited hydropericardium nor was abdominal or subcutaneous edema observed. Amyloid deposits were not detected by Congo red stain.

Prestt et al. (1970), using the Bengalese finch, reported that kidneys were larger in birds that died from PCB than in controls. Flick et al. (1965), using chickens, mentioned both enlarged adrenals and kidneys from PCB treatment. In the present study, kidneys were larger in pheasants given 10 or 20 mg daily, but neither kidney nor adrenal enlargement was visually detected during necropsy. McCune et al. (1962), using Aroclor 1242 with chickens, mentioned both enlarged livers and kidneys in birds given PCB. Platonow and Funnell (1971) and Rehfeld (1971), using 1-day-old chicks, reported enlarged livers with dietary intake of Aroclor 1254. Grant et al. (1971) reported enlarged livers and decreased spleen size over a period of time in the rat. Flick et al. (1965) and Vos and Koeman (1970) reported small spleens in their studies with chickens. Although hydropericardium was found in varying degrees by many of the authors cited, it was found only rarely in the present study. Vos and Koeman (1970) found that Phenoclor DP 6 and Clophen A60 caused much more liver necrosis and hydropericardium than Aroclor 1260. This was probably due to contaminants (Vos et al. 1970).

PCB in Combination With Dieldrin

Mortality among pheasants of both sexes that were 6 to 9 months old when they were given dieldrin, PCB, or a combination of the two varied with the level of chemical administered (Table 16). Among the 22 pheasants on each level, 3 died with 4 mg dieldrin per capsule and 6 died with 8 mg dieldrin. The same proportions held true for PCB, since 4 died with 50 mg PCB per capsule and 9 died with 100 mg PCB. When 50 mg PCB and 4 mg dieldrin were administered together, a total of 9 birds died. None of 11 control birds died during this period of time. These data suggest that effects of PCB and dieldrin together are additive, not synergistic. Heath et al. (1970) found that the joint toxicity of Aroclor 1254 and DDE given to Japanese quail was additive and found no evidence of synergism in their joint effect.

Residues in Wild Birds

A pooled sample of six pheasant livers taken from wild South Dakota pheasants had <0.1 ppm PCB. A pooled sample of three livers collected near Washington, Pennsylvania, had <0.1 ppm PCB, and a pooled sample of six livers collected southeast of Lancaster, Pennsylvania, had 2.0 ppm PCB. A pooled sample of six livers collected east of Gary, Indiana, had 0.5 ppm PCB, and a pooled sample of six livers taken from pheasants in Benton County, Indiana, had 1.5 ppm PCB.

These relatively low levels in livers of wild pheasants taken both from industrial areas and rural areas indicate these pheasants

Table 16. Mortality occurring among 22 pheasants of both sexes that were 6 to 9 months of age when PCB and dieldrin were administered separately and in combination. No mortality occurred among 11 control birds.

Group Treatment	No. of Deaths in Week					Total Deaths
	1	2	3	4	5	
50 mg PCB	1	0	0	1	2	4
100 mg PCB	0	3	0	4	2[a]	9
4 mg dieldrin	3	0	0	0	0	3
8 mg dieldrin	3	0	0	1	2	6
50 mg PCB and 4 mg dieldrin	1	2	2	0	4	9

[a] These two birds were near death at the end of the experiment.

were probably exposed to small amounts of PCB. Prestt et al. (1970) found that residue levels in birds in Britain were related to their food habits. They found the most PCB in livers of fresh-water fish-eating birds (up to about 900 ppm); bird-feeding raptors had up to 70 ppm; birds which eat mammals had up to 50 ppm; birds with a mixed diet of mammals, birds, and carrion had up to 15 ppm; and those which eat insects had 0 to 1 ppm.

SUMMARY AND CONCLUSIONS

PCB given to laying pheasant hens adversely affected egg production, hatchability, and viability of the embryo about the time of hatching but did not affect fertility or eggshell thickness. A subtle effect on behavior was indicated by studies on the visual cliff and of the ability of offspring to avoid hand capture. These behavioral effects have been reported for birds with organochlorine insecticides and could be deleterious to a wild species in that instinctive patterns necessary for survival are involved. Behavior may be affected through PCB administration to the cock as well as through the hen, as shown by the study of hand catching. This implies that the effect is, though unexplained as to mechanism, more than through the physical presence of the PCB in the egg-yolk lipids. The presence of PCB in egg lipids is probably responsible for the observed effect of increased mortality in young during the first 6 weeks of life and in a possible depression of weight at 6 weeks of age.

Large doses of PCB, 210 mg daily, effected a loss of appetite, but lesser doses did not affect feed consumption until just prior to death. The death of birds given PCB was not attributable to the starvation that occurred in the few days prior to death, because birds dying from PCB did not lose as much weight as birds that were not given PCB but were starved. Further, starved birds did not show the degeneration of liver cord cells and depletion of lymphatic nodules in the spleen.

PCB were rapidly and readily absorbed into the pheasant's body, stored in the lipid fraction, and excreted slowly in feces and eggs. Excretion via the egg may be an important means of ridding the body of PCB for the hen, but the PCB in the egg yolk lipid may be dangerous for the offspring because of altered behavior and lowered survival both of the embryo and hatched young.

Brain tissue residues may be a valuable indicator of PCB toxicosis. Levels of 300 to 400 ppm or more were associated with death due to PCB. Marked splenic atrophy was the most consistent characteristic noted visually among several organ parameters checked in birds that died from PCB. Enlarged kidneys and livers were also useful characters in attempting to diagnose PCB toxicosis.

PCB and dieldrin were not, when combined, synergistic in their joint toxicity to pheasants.

Livers from wild pheasants collected in Pennsylvania, Indiana, and South Dakota did not exceed 2 ppm PCB, indicating relatively low-level contamination.

Since PCB were found to have many of the characteristics of organochlorine insecticides and exhibit the same effects, they are environmental contaminants important to producers of animal food products. They may appear in the finished product (i.e., meat, milk, or eggs) and are thus economically important.

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