PCB - Literature

Effects of Polychlorinated Biphenyls on Birds

by

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ABSTRACT

Toxicities of six technical polychlorinated biphenyl (PCB) compounds (Aroclor[1/] 1232, 1242, 1248, 1254, 1260, and 1262) were studied on penned mallards (Anas platyrhynchos), pheasants (Phasianus colchicus), bobwhite (Colinus virginianus), and Japanese quail (Coturnix coturnix) at the Patuxent Wildlife Research Center, Laurel, Maryland. LC_{50}'s expressed as ppm of PCB in dry feed, were determined for 2-week-old birds fed treated diets for 5 days. Aroclor toxicity, generally less than that of DDT, was found to be positively correlated with chlorine percentage (last two digits of Aroclor number). The joint toxicity of Aroclor 1254 and DDE on Japanese quail was additive, not synergistic. Low dietary levels (25 and 50 ppm) of Aroclor 1254 produced no measurable reproductive effects in mallards and bobwhite.

[1/] Registered name, Monsanto Company

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Polychlorinated biphenyl (PCB) compounds are a group of synthetic chlorinated hydrocarbons that have been used industrially in tank car quantities since the early 1930's (Penning, 1930). They are marketed as complex mixtures[1/] which function, for example, as plasticizers in diverse materials, as electrical insulators and impregnators, as grinding and cutting oil, hydraulic fluids, and high temperature lubricants, and as heat exchange media (ref. 22). PCB's are rated in industry as highly toxic compounds and can cause permanent or fatal liver damage (Sax, 1963).

In 1966, PCB's were identified as environmental contaminants when Jensen (1966) reported residues in Swedish pike, an eagle, and human hair. Identification was confirmed by mass spectrometry.

Subsequent reports of residues in numerous terrestrial and aquatic forms have established the global ubiquity of PCB's. They have been reported in British wildlife (Holmes et al., 1967; Prestt et al., 1970), in Scottish and Canadian seals and porpoises (Holden and Marsden, 1967), in birds and fish from California and the Pacific (Risebrough et al., 1968), in fish, mussels, and birds of the Netherlands (Koeman et al., 1969),

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[1/] Registered names include Aroclor, Clophen, and Phenochlor

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in North American cormorants and pelicans (Anderson et al., 1969), in marine animals of Sweden (Jensen et al., 1969), and in North American bald eagles (Bagley et al., 1970).

In both environment and laboratory, PCB's behave like DDT and other organochlorine pesticides. Poorly soluble in water but readily so in fats, they concentrate in animal lipids together with pesticidal residues. As with DDT, concentrations tend to increase through trophic levels of food chains (Jensen et al., 1969).

PCB's are recovered and detected by the same analytical methods used for organochlorine pesticides, and chemists have necessarily developed procedures to separate them prior to quantification (Koeman et al., 1969; Bagley et al., 1970). Before 1966, PCB's were either misidentified as specific pesticides or viewed as unidentified compounds, possibly pesticidal metabolites (Holmes, et al., 1967); however, detectable quantities have been in the environment at least since 1944 (Jensen, 1966).

Several routes of entry into the environment have been postulated. For example industrial leakage, flushing, or dumping of waste PCB's are conceivable, and refuse burning could release them from plastic materials into the atmosphere.

The ecological significance of PCB contamination is virtually uninvestigated. Being toxic compounds they must be classified as potentially hazardous, but toxicological information is limited. Their direct environmental association with several pesticides makes synergistic actions conceivable.

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Studies to determine effects of PCB's on birds were initiated at the Patuxent Wildlife Research Center in 1968. From these studies we report the following determinations:

1. Median lethal concentrations (LC_{50}'s) of six technical PCB mixtures (Aroclor 1232[1/], 1242, 1248, 1254, 1260, and 1262) in diets of 2-week-old mallards (Anas platyrhynchos), pheasants (Phasianus colchicus), bobwhite (Colinus virginianus), and Japanese quail (Coturnix coturnix).
2. Joint actions of three ratios of Aroclor 1254 and DDE combined in diets of Japanese quail for 5 days.
3. Reproductive effects of sublethal dietary levels of Aroclor 1254 on mallards and bobwhite.

DIETARY TOXICITIES OF PCB'S

Toxicities of individual Aroclors:

Dietary LC_{50}'s were derived to assess the relative toxicities of the different Aroclors and to compare the toxicities with those of several common organochlorine pesticides. The LC_{50}'s also serve to rank the sensitivities of the four species to each chemical. An LC_{50} is defined here as ppm of a chemical in an ad libitum diet expected to produce 50 percent mortality among 2-week-old birds in 8 days, 5 days of toxic diet followed by 3 days of untreated diet (the latter included to detect mortality induced beyond the dosage period).

The test protocol was that described by Heath and L. Stickel (1965). Briefly, chemical was introduced as a dietary component

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[1/] Last two digits of Aroclor numbers denote chlorine percentage

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because ingestion is seemingly the principal route of exposure in the wild. Aroclors and pesticides were dissolved in corn oil and then mixed thoroughly with dry feed in a ratio of 2 parts of solution to 98 parts of feed. An equal amount of pure corn oil was added to control diets.

To standardize experimental conditions, all Aroclors were tested simultaneously on a given species in a completely randomized design. Each LC_{50} was derived from a group of 60 birds placed in six pens of 10 each. One of six dietary levels was assigned to each pen. Levels were spaced geometrically over a range expected to produce from 10 to 90 percent mortality. Pens of control birds were used to adjust LC_{50}'s for extraneous mortality. LC_{50}'s were computed by methods of probit analysis (Finney, 1952).

Table 1 presents LC_{50}'s, with 95 percent confidence limits, of Aroclors, DDE, DDT, dieldrin, and endrin for the four test species. These determinations show that dietary toxicities of Aroclors were, with few exceptions, less than those of the four organochlorine pesticides. Endrin was approximately 100 times, and dieldrin 10 times, as toxic as any Aroclor; and DDT was as much as 4 times as toxic. Aroclors were somewhat more toxic to mallards than DDE, as were the highly-chlorinated Aroclors to bobwhite; otherwise, DDE was more toxic than PCB's. Presst et al. (1970) found Aroclor 1254 to have 1/13 the lethal toxicity of DDT to adult Bengalese finches (Lonchura striata) in a 56-day feeding study.

There was a positive relationship between the percentage of chlorine in a technical Aroclor and its toxicity. The relationship held true for those containing less than 60 percent chlorine, Aroclors 1260 and 1262 deviating slightly. Mallards were relatively less

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responsive to chlorine content than the three gallinaceous species.

There were consistent species differences in sensitivity to Aroclors. Bobwhite were most sensitive, followed in turn by pheasants, mallards, and Japanese quail. LC_{50}'s for Japanese quail (the most tolerant species) were from 3 to 4 times as high as for bobwhite. Mallards were about twice as tolerant as pheasants, which were nearly as sensitive as bobwhite. The order of species sensitivity to the organochlorine pesticides was somewhat different; mallards were from 2 to 4 times as tolerant as the gallinaceous species, the sensitivities of the latter being relatively similar.

During PCB poisoning, birds became lethargic and tended to assume a crouching position within the final 24 hours of life. They displayed mild tremors during the final hour, tremors being less violent than those associated with DDT poisoning.

Autopsies of eight bobwhite chicks poisoned by Aroclor 1254 revealed a general decline in physical condition accompanied by a complete absence of fat, and there was excessive fluid in the pericardial sac. Similar effects from Aroclor 1242 were reported in Vantress-White Rock, cross-bred chicks (McCune et al., 1962). Vos and Koeman (1970) describe detailed pathological changes in Hubbart cockerels fed diets with 400 ppm of one of three 60 percent-chlorinated commercial PCB preparations.

Joint toxicity of Aroclor 1254 and DDE:

Using the above 8-day protocol, Japanese quail were fed diets containing combinations of Aroclor 1254 and DDE to test the possibility of synergistic action. Three ratios of the chemicals were tested, one combining 50 percent of the known effective dosages of both chemicals, one combining 90 percent of an effective dosage of Aroclor with

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10 percent of that of DDE, and one combining 10 percent of an effective dosage of Aroclor with 90 percent of that of DDE. Both chemicals were also administered separately as is necessary in tests of joint action. Analysis was by the method of Wadley (1945).

All joint actions proved to be essentially additive. In terms of effective dosages the 50:50 percent mixture was about 91 percent as toxic as the individual chemicals, the 90 percent Aroclor:10 percent DDE was 98 percent as toxic, and the 10 percent Aroclor:90 percent DDE was 95 percent as toxic. Clearly there was no suggestion of synergism.

REPRODUCTIVE EFFECTS OF PCB'S

Two-year studies to determine effects of Aroclor 1254 on mallard and bobwhite reproduction began at the Center in 1968. DDE was included as a comparative treatment because of its close environmental association with PCB's and because of its capacity to impair mallard reproduction (Heath et al., 1969). A test for synergistic effects on reproduction using a mixture of Aroclor 1254 and DDE was necessarily confined to the bobwhite study.

Chemicals were administered in the diet. Mallard treatments included dietary concentrations of Aroclor 1254 at 25 ppm and DDE at 10 ppm. Bobwhite received Aroclor 1254 at 50 ppm, DDE at 30 ppm, and a mixture of 25 ppm Aroclor 1254 plus 15 ppm DDE. Diets were prepared by dissolving chemicals in corn oil and mixing one part of oil solution with 99 parts of dry commercial feed.

Test birds had been hatched in incubators, the progeny of captive breeders phenotypically indistinguishable from wild birds. Mallards

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were housed in wooden pens constructed on concrete slabs; pens were spread with straw and supplied with troughs of running water. Bobwhite were maintained outdoors in wire-floored cages.

A completely randomized design was used throughout both studies. Five hens and two drakes were allotted to each mallard pen, and each treatment was replicated in four pens. Bobwhite cages were assigned three hens and two cocks; each treatment was replicated in six cages.

Mallards received treated diet ad libitum from about 11 weeks before their first laying season through their second year of laying. A similar schedule was initiated with bobwhite; however, heavy mortality prior to the second breeding season limited the study to a single year.

All eggs were collected daily for 8 weeks each season and stored at 16° C and 55 percent relative humidity. At intervals of 2 weeks they were examined for cracked shells, incubated, and candled after 2 and 3 weeks of incubation to measure embryonation and embryo survival. Hatchlings were leg-banded for identification with parental pen and observed on diet free of chemical for 14 days. Data were recorded for each pen for every 2-week set of eggs. From these data we calculated the seasonal parameters of reproductive success presented in Tables 2 and 3. Measurements were analyzed by weighted analysis of variance, pen statistics being weighted according to the number of eggs or hatchlings on which they were based. Angular transformations were applied to percentages for analysis, and means were separated by methods of Duncan (1955) and Kramer (1956).

Prior to incubation eggs with sound shells from alternate 2-week sets were sampled to measure shell thickness. Mean thicknesses of dry shell,

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with membrane, are presented in Tables 4 and 5.

Effects on mallard reproduction:

Aroclor 1254 did not produce statistically significant effects on mallard reproduction at the 95 percent level of confidence (Table 2). The apparent reduction in egg production, and subsequently in ducklings per hen, resulted from one pen of breeders failing to lay normally; however, egg production in the remaining Aroclor pens did not differ markedly from that in control pens. Other reproductive determinations differed little between control and Aroclor treatments. In particular, Aroclor caused no detectable increase in eggshell cracking or reduction in shell thickness; DDE induced significant shell thinning and cracking and a reduction in hatching success, as was reported in earlier work (Heath et al., 1969).

Effects on bobwhite reproduction:

Bobwhite reproduction after 1 to 3 months of dosage was not measurably affected by either Aroclor 1254 or DDE, administered singly or in combination (Table 3). Numerically, mean egg production was lowest, and percentages of cracked eggs highest, in control birds, although differences were not substantiated at the 5 percent level of significance. The suggested decrease in chick survival in all experimental groups was not significant.

Effects on eggshell thickness:

A preliminary investigation of PCB effects on mallard eggshell thickness was conducted in 1968. Single groups of year-old birds comprised of eight hens and one drake were fed diets containing 0, 10, or 500 ppm of Aroclor 1254 from March 21 to April 29. Eggs were collected

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from March 22 to April 11 (21 days) and again from April 25 to 29 (5 days). Eggs were opened and shells dried with forced air for 16 hours at 27° C. Shell thickness was measured at the waist with a Starrett dial gage graduated in 0.01 mm units.

Average shell thicknesses did not differ significantly among treatments during the first collection period. Mean thicknesses from the 0, 10, and 500 ppm Aroclor treatments were 0.357, 0.348, and 0.354 mm in samples of 41, 36, and 27 eggs, respectively. During the second period mean thicknesses were 0.357, 0.370, and 0.378 mm in 18, 30, and 17 eggs. It can not be determined if the 6 percent increases in mean shell thickness in both Aroclor 1254 treatments are real, since eggs could not be identified with individual hens, and pen replication was lacking. Certainly the Aroclor 1254 treatments did not induce shell thinning.

Analysis of shell thickness measurements from the 2-year mallard study, in which biweekly pen averages served as sampling units in a split-plot design, failed to indicate thickness changes from Aroclor 1254 at 25 ppm (Table 4). DDE at 10 ppm induced significant shell thinning both years ($P < 0.05$). The numerical increase in Aroclor 1254 shells the second year relative to decreases in control and DDE shells (years X treatments interaction) was not significant.

Analysis of variance of bobwhite eggshell data revealed no measurable differences in mean shell thickness among treatments (Table 5). Of particular note is the fact that DDE induced eggshell thinning in mallards but not in bobwhite.

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PCB residues in mallard eggs:

Two second-year mallard eggs from the Aroclor 1254 treatment contained 56 ppm and 33 ppm of PCB, wet weight. Measurement was by thin layer chromatography (B. M. Mulhern et al., 1971). Methods of cleanup were essentially those described by Reichel et al. (1969).

The egg residues were also analyzed by gas-liquid chromatography (GLC) using an OV-17 column. Comparison of the GLC chromatograms of the mallard eggs with an Aroclor 1254 standard reveals that early peaks from the egg are greatly diminished (Figure 1). A similar result was reported in brains and livers of Japanese quail (Koeman et al., 1969). The differences may be due to differential absorption of PCB's in the system or to metabolism of low-chlorine molecules. A positive relationship has been shown between the number of chlorine atoms per PCB molecule and GLC retention time (Koeman et al., op. cit.; Bagley, et al., 1970).

Chronic effects of PCB's on breeders:

There were no deaths among 19 hen and 8 drake mallards fed diets with 25 ppm Aroclor 1254 from December 19, 1968, to May 6, 1970. There was complete survival among control birds during this period, although two of 20 hens and one of 8 drakes on 10 ppm DDE treatment died prior to the second breeding season. As mentioned earlier, bobwhite suffered heavy mortality prior to the second breeding season, and the study was terminated.

Reproductive effects of PCB's in chickens:

In a study conducted for the Monsanto Company (ref. 9), significant reproductive impairment in White Leghorn chickens (Gallus gallus) fed Aroclor 1242 at 10 or 100 ppm or Aroclor 1254 at 100 ppm was demonstrated. The dosages reduced egg production and hatchability and caused thin eggshells. There were no measurable effects from Aroclor 1242 at 1 ppm.

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from Aroclor 1254 at 1 or 10 ppm, or from Aroclor 1260 at 1, 10, or 100 ppm.

DISCUSSION AND SUMMARY

It is encouraging that the lethal toxicity of PCB compounds proved to be less than that of DDT to the species investigated. However, the four-fold difference in sensitivity to PCB's observed between two gallinaceous species indicates that there may be species much more sensitive than those tested.

Aroclor 1254 did not induce measurable changes in eggshells or in reproductive success of mallards at 25 ppm or bobwhite at 50 ppm; however the material did induce such changes in White Leghorns at 100 ppm (a severe dosage) but not at 10 ppm. Unfortunately the findings do not permit a valid comparison of sensitivity to Aroclor 1254 between White Leghorns and mallards or bobwhite. The fact that Aroclor 1242 affected reproduction at 10 ppm, while Aroclor 1254 did not, suggests effects from specific PCB isomers not necessarily related to chlorine content. Eggshell thinning from PCB's has been suggested by other investigators (Anderson et al., 1969; Peakall, 1970).

There are, of course, many aspects of PCB toxicity not investigated in this study. Extensive, integrated research will be required to assess fully the effects of polychlorinated biphenyls on birds.

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Table 1. Toxicity comparisons of Aroclors (polychlorinated biphenyls) and four organochlorine pesticides in diets of 2-week-old birds

Chemical	LC_{50}'s[1], with 95% Confidence Limits Parenthesized			
	Mallard	Pheasant	Bobwhite	Japanese Quail
Aroclor 1232[2]	--	3150 (2625-3950)	3000 (2575-3500)	>5000
Aroclor 1242	3180 (2610-3880)	2080 (1840-2350)	2100 (1705-2610)	>5000
Aroclor 1248	2795 (2265-3420)	1310 (1165-1480)	1175 (965-1440)	4845 (4355-5410)
Aroclor 1254	2700 (2160-3310)	1090 (965-1230)	605 (410-840)	2900 (2600-3240)
Aroclor 1260	1975 (1365-2750)	1260 (1105-1435)	745 (575-935)	2185 (1915-2480)
Aroclor 1262	3010 (2460-3635)	1235 (1085-1405)	870 (700-1070)	2290 (2040-2575)
DDE	3570 (2810-4670)	840 (730-970)	825 (695-980)	1355 (1110-1650)
DDT	1870 (1500-2370)	310 (255-375)	610 (515-725)	570 (470-690)
dieldrin	200 (160-240)	55 (49-61)	39 (33-45)	56 (51-61)
endrin	22 (17-31)	14 (10.5-17.5)	15 (10.5-24)	15 (12.5-17)

[1] LC_{50}: ppm chemical in *ad libitum* diet expected to produce 50% mortality in 8 days, 5 days of toxic diet followed by 3 days of untreated diet. Each determination based on 60 birds.

[2] Last two digits of Aroclor numbers denote chlorine percentage by weight.

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Table 2. Reproductive success among penned mallards maintained for two seasons[1] on feeds containing Aroclor 1254 or DDE.

	Season	Chemical added to feed (ppm) Control	Aroclor 1254 (25)	DDE (10)
Pens of birds (5 ♀♀, 2 ♂♂ each)	1969	4	4	4
	1970	4	4	4
Hen-seasons (8 weeks each)	1969	20	20	19.1
	1970	20	19	18
Total eggs laid	1969	590	433	628
	1970	630	468	632
Eggs laid per hen-seasons	1969	29.5	21.7	32.8
	1970	31.5	24.6	35.1
Eggs cracked (%)	1969	4	6	20[2]
	1970	9	9	28[2]
Eggs embryonated of eggs set (%)	1969	86	94	80
	1970	88	90	68
Embryos alive at 3 weeks (%)	1969	98	96	94
	1970	92	92	88
Normal hatchlings of 3-week embryos (%)	1969	70	63	47[2]
	1970	59	64	53
Normal hatchlings alive at 14 days (%)	1969	94	91	97
	1970	93	96	92
14-day ducklings of embryonated eggs (%)	1969	64	55	43[2]
	1970	51	57	43[2]
14-day ducklings per hen-seasons	1969	13.0	8.8	7.8[2]
	1970	11.6	10.4	6.7[2]

[1] Dosage started December 19, 1968; eggs collected from March 12 through May 6, 1969, and from March 18 through May 12, 1970.

[2] Differences from controls statistically significant ($P < 0.05$).

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Table 3. Reproductive success among penned bobwhite fed diets containing Aroclor 1254, DDE, or a mixture of both chemicals.[1/]

	Chemical added to feed (ppm)			
	Control	Aroclor 1254 (50)	DDE (30)	Aroclor 1254 + DDE (25) + (15)
Pens of birds (3 ♀♀, 2 ♂♂ each)	6	6	6	5
Hen-seasons (8 weeks each)	16.6	16.4	15.3	14.9
Total eggs laid	347	368	415	386
Eggs laid per hen-season	20.9	22.4	27.1	26.0
Eggs cracked (%)	10	7	5	7
Eggs embryonated of eggs set (%)	88	90	93	93
Embryos alive at 3 weeks (%)	97	97	98	97
Normal hatchlings of 3-week embryos (%)	96	91	96	92
Normal hatchlings alive after 14 days (%)	78	72	70	71
14-day chicks of embryonated eggs (%)	73	64	66	63
14-day chicks per hen-season	11.5	11.5	15.1	13.5

1/ Dosage started April 3, 1968; eggs collected from April 27 through June 21, 1968.

None of the above differences was significant at the 95 percent level of confidence.

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Table 4. Mean thickness of eggshells of penned mallards maintained through two seasons on feed containing Aroclor 1254 or DDE.

		Chemical added to feed (ppm)		
	Season	None	Aroclor 1254 (25)	DDE (10)
No. of eggs	1969	28	27	27
	1970	32	17	26
Shell thickness (mm)	1969	0.375	0.360	0.339[1/]
	1970	0.365	0.368	0.328[1/]

[1/] Differences from controls statistically significant ($P < 0.05$)

Table 5. Mean thickness of eggshells of penned bobwhite maintained through one season on feed containing Aroclor 1254, DDE, or a mixture of both chemicals.

	Chemical added to feed (ppm)			
	None	Aroclor 1254 (50)	DDE (30)	Aroclor 1254 + DDE (25) + (15)
No. of eggs	14	17	20	18
Shell thickness (mm)	0.207	0.208	0.205	0.215

None of the above differences was significant at the 95 percent level of confidence.

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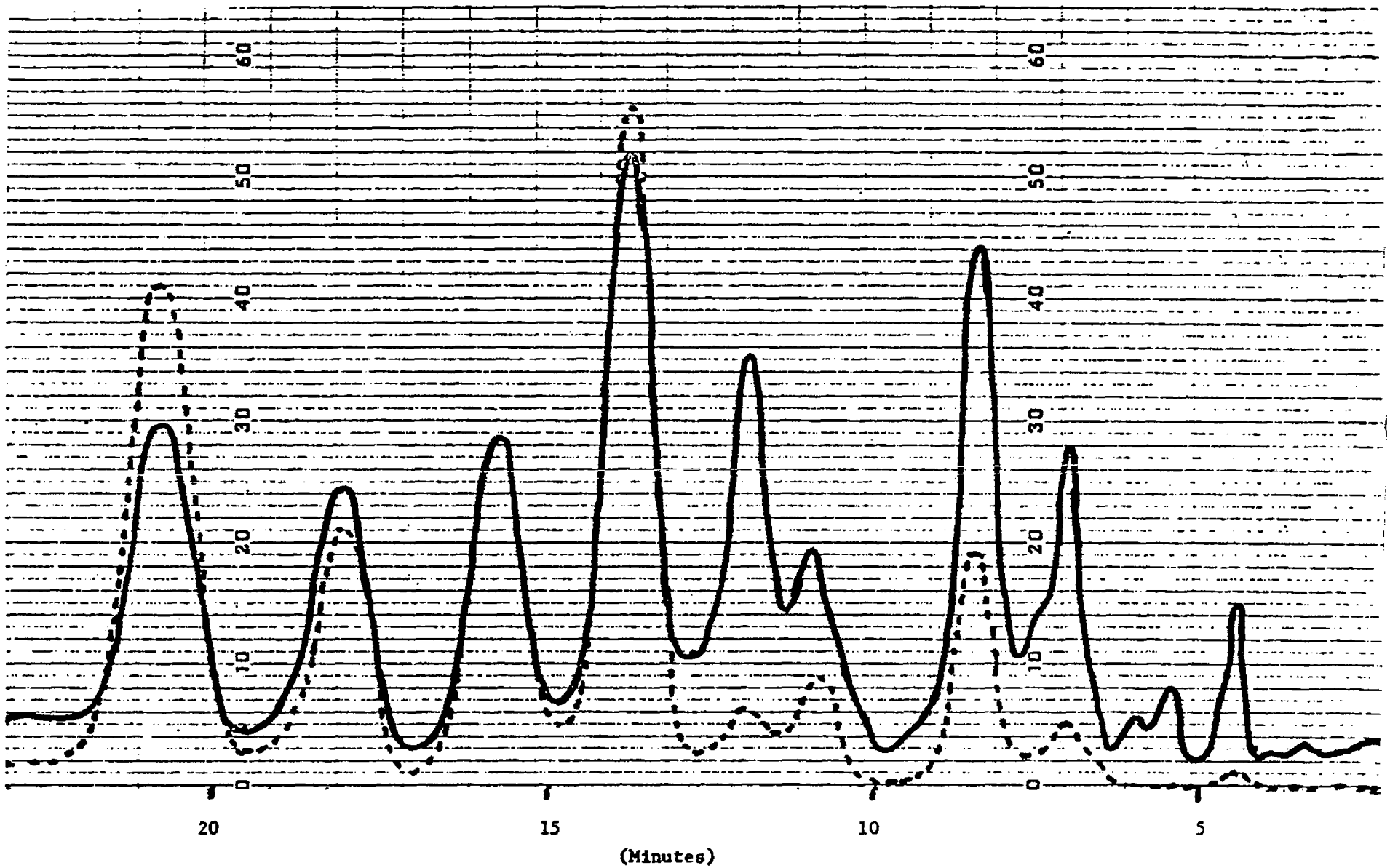


Figure 1. Gas chromatograms of technical Aroclor 1254 (solid line) and Aroclor 1254 residues from a mallard egg (dotted line) of a hen maintained on feed containing 25 ppm.

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APPENDIX

The chemical names of compounds mentioned in this paper are:

DDE 1,1-dichloro-2,2-bis (p-chlorphenyl) ethylene

DDT 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane

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

Endrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8, 8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene

PCB Polychlorinated biphenyl

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