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ELIMINATION PATTERN OF AROCLOR 1254 COMPONENTS IN THE BOBWHITE

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SUMMARY

The gas–liquid chromatographic profile for Aroclor 1254 did not maintain its integrity in bobwhite quail fed Aroclor 1254 for 14 days and clean food 14 or 42 days thereafter. Absorption of all components occurred at essentially the same rate, as shown by analysis of quail sacrificed within an hour after a single oral dose of Aroclor. Acetonitrile–hexane partitioning altered the peak pattern of the Aroclor 1254 standard.

Significant alteration of certain polychlorinated biphenyl isomers appeared to take place. Dechlorination was clearly apparent, and isomeric transformation is suggested.

INTRODUCTION

The presence of polychlorinated biphenyls (PCBs) in the environment, their toxicity, and some of their physiological effects have been described for various species.

The PCBs are industrial compounds marketed in the United States under the trade name Aroclor (Monsanto). Other registered names are Clophen and Phenochlor. They all contain a complex mixture of closely related chlorinated biphenyls varying in chlorine content. Aroclor 1254, which was used in this study, contains 54% chlorine[1] as indicated by the last two digits in its numerical designation. Some other Aroclors are designated 1232, 1242, 1248, 1260, and 1262. Their uses are numerous and varied. There are 210 possible isomers and 102 most likely[2]. BAGLEY *et al.*[3] identified 18 isomers in Aroclor 1254 by gas-liquid chromatography–mass spectrometry (GLC–MS). KOEMAN *et al.*[4] showed the presence of 11 isomers in Aroclor 1260, and SISSONS AND WELTI[5] have shown that 69 compounds are present in Aroclor 1254.

Analysis of PCB's is routinely accomplished by GLC with electron capture

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detection. The commonly occurring chlorinated pesticides such as DDT and its metabolites are determined similarly, and as both occur in fish and wildlife tissues, the PCBs must be separated in order to obtain reliable analytical data. Several methods have been used[6–8].

There has been less progress in quantitation of PCBs and perhaps there are as many methods used as there are analysts. The problems of quantitation are related to the complexity of the closely related components of the Aroclors. In our laboratory, we use a semiquantitative thin-layer chromatographic procedure as reported by MULHERN *et al.*[9]. This method is applicable to all Aroclors in the 1200 series. Other investigators have used GLC peak heights of several peaks and even a single peak. ROTE AND MURPHY[10] used a detector-response curve to determine the amount of each chlorinated component in the PCB standards. A review of various methods for quantitating PCB was presented by PEAKALL AND LINCER[11].

In analysis of bobwhite *(Colinus virginianus)* given a treated diet of Aroclor 1254, we observed that the ratios of the Aroclor peaks to each other differed from those in the Aroclor 1254 standard. Some peaks were relatively higher and others lower than in the standard. We set up the present study to observe elimination patterns of Aroclor 1254 components in relation to time. Changes in relative peak heights can greatly influence the value of a quantitative method, for if the integrity of the Aroclor GLC profile is not maintained, then only a method, which accounts for these changes, will give quantitatively dependable results.

EXPERIMENTAL

Diet treatment of bobwhite

Aroclor 1254 was dissolved in corn oil and then mixed with dry feed in a ratio of 2 parts of solution to 98 parts of feed. The concentration of Aroclor 1254 in solution was such that, when mixed with feed, a diet containing 300 p.p.m. of Aroclor resulted.

Early in May 1970, 30 bobwhite, 1-year-old males, raised at the Patuxent Wildlife Research Center, were placed in 6 cages (5 birds per cage) and maintained on turkey starter mash for 1 week prior to start of treatment. Following 1 week of acclimation, 3 cages of birds were provided *ad libitum* access to the treated diet and 3 cages were provided the same commercial diet free of toxicant but containing 2 parts of corn oil to 98 parts of feed. On day 14 after start of treatment 3 birds were sacrificed. The remaining birds were then placed on clean feed; 3 were sacrificed on day 28 and the remaining 9 on day 56. Control birds were sacrificed at the same rate and interval as treated birds. At the end of test, 3 birds from the control group were each given orally 1 capsule dose of Aroclor 1254 in corn oil equivalent to 500 mg/kg body weight and were sacrificed after 1 h. All birds, except those sacrificed, survived the test period.

Preparation of sample

The carcass (after removal of skin, wings, liver, feet, and gastrointestinal tract) was ground and mixed in a Hobart food cutter. A 30-g aliquot was mixed with sodium sulfate and extracted 7 h with redistilled hexane in a Soxhlet apparatus.

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Whole livers, which weighed approx. 3–4 g, were ground in a Waring blender along with sodium sulfate and extracted. Extracts were cleaned up by acetonitrile–hexane partitioning and eluted on a Florisil column as previously described[12]. A 100-g sample of the untreated feed was extracted and cleaned up by the same procedure and analyzed by GLC.

Apparatus

An LKB Model 9000 GLC–MS apparatus was used for analysis. The spiral glass column (9 ft. × 0.25 in.) was packed with 5% OV-17 on 60–80 mesh Gas-Chrom Q. Carrier gas was helium, flowing at a rate of 40 ml/min. Operating temperatures were: flash heater, 230°; GLC oven, 220°; separator, 240°; and ion source, 290°. The ionization potential was 70 eV, trap current 60 μA, and accelerating voltage 3.5 kV.

GLC–MS analysis

Total ion current (TIC) chromatograms were made for two birds from each group sacrificed, using a 30-g aliquot sample extract made to equal volume. The volume of sample injected was varied in order to maintain the height of peak No. 5 at approximately the same level in all chromatograms. This level was arbitrarily set at the level produced by 6 μg of Aroclor 1254 standard. The amounts of sample injected were as follows: 30 mg of the original sample of the capsule-dosed birds; 109 mg of the sample of birds fed treated diet for 14 days; and 90 mg of the samples of the birds fed treated diet for 14 days and untreated feed for either 14 or 42 days thereafter.

To evaluate possible effects of the clean-up procedure on the Aroclor standard, 300 μg of technical Aroclor 1254 was partitioned by acetonitrile–hexane and eluted on a Florisil column in the identical procedure, used for the bird tissues. The eluate was made to appropriate volume and a portion was injected into the GLC–MS apparatus to produce a response on the TIC chromatogram approximating 6 μg of Aroclor 1254 standard.

Relative peak heights

Relative peak heights in TIC chromatograms were calculated by choosing peak No. 5 as reference and assigning it a value of 100. This was almost always the highest peak, and its relative depreciation appeared orderly. All peaks were maintained within the linear range of the recorder, and the height of the reference peak in each chromatogram was kept similar by changing the amount of the sample injected, as described above.

RESULTS AND DISCUSSION

Results are shown in Table I and in Figs. 1a–f. Table I compares the relative peaks heights in the Aroclor 1254 standard, the Aroclor 1254 standard subjected to acetonitrile–hexane partitioning, and the quail carcass extracts. The change in the Aroclor as a result of partitioning is believed to be due to the distribution coefficient effect of acetonitrile–hexane. STALLING *et al.*[13] also observed this effect in their work and determined f values for all peaks in Aroclor 1254.

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TABLE I

RELATIVE PEAK HEIGHTS[a] IN TIC CHROMATOGRAMS

Sample	Peak No.											
	1	2	3	4	5	6	7	8	9	10	11	12
R_x[b]	0.49	0.58	0.69	0.78	0.83	0.94	1.06	1.13	1.27	1.40	1.61	1.81
No. of Cl[c]	4	4	4.5	5	5	5	5	6	6	6	6	6
Aroclor[d] 1254 (S)	101	34	49	80	100	23	39	68	73	30	30	34
Aroclor[e] 1254 (P[f])	125	48	57	98	100	32	44	71	65	27	31	31
Extract C[g]	118	48	58	96	100	32	49	73	70	28	33	33
Extract D[g]	93	15	25	45	100	0	39	51	63	28	22	39
Extract E[h]	54	0	84	0	100	0	33	23	63	36	12	48
Extract F[i]	25	0	26	0	100	0	34	0	77	66	14	86

[a] Peak No. 5 = 100%.
[b] p,p'-DDE = 1.0.
[c] No. of Cl in PCB.
[d] S = standard.
[e] P = standard partitioned by acetonitrile–hexane and eluted on Florisil.
[f] Carcass of quail administered 1 capsule of Aroclor 1254, 500 mg/kg body weight. For details, see text.
[g] Carcass of quail sacrificed at day 14. For details, see text.
[h] Carcass of quail sacrificed at day 28. For details, see text.
[i] Carcass of quail sacrificed at day 56. For details, see text.

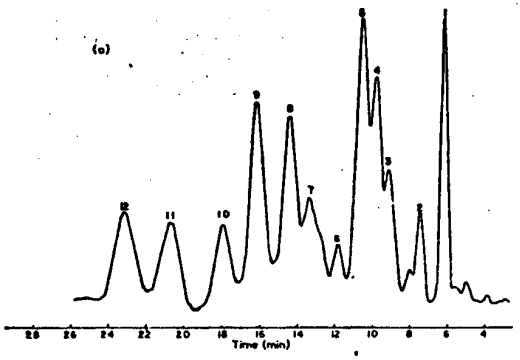

Fig. 1. TIC chromatograms. GLC conditions: 9 ft. × 25 in. column, 5% OV-17; oven temperature, 220°, and helium flow-rate, 40 ml/min. (a) Aroclor 1254 standard; (b) Aroclor 1254, acetonitrile–hexane partitioned, standard; (c) carcass extracts of quail administered a single capsule dose of Aroclor 1254 at 500 mg/kg body weight; (d) carcass extracts of quail sacrificed after 14 days' dietary dosage at 100 p.p.m. of Aroclor 1254; (e) carcass extracts of quail sacrificed after 14 days' dietary dosage at 300 p.p.m. of Aroclor 1254 followed by 14 days of untreated food; (f) carcass extracts of quail sacrificed after 14 days' dietary dosage of 300 p.p.m. of Aroclor 1254 followed by 42 days of untreated food.

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	11	12
.40	1.61	1.81
	6	6
	30	34
	31	31
	33	33
	22	39
	12	48
	14	86

or details, see text.

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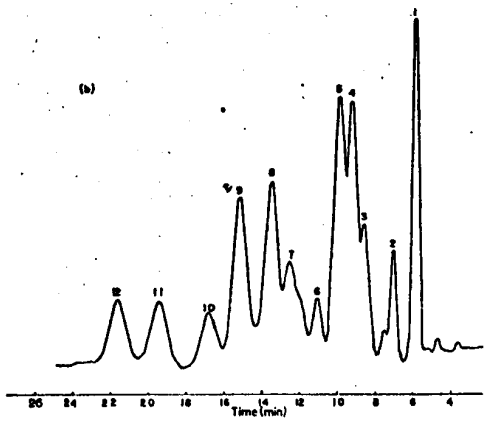

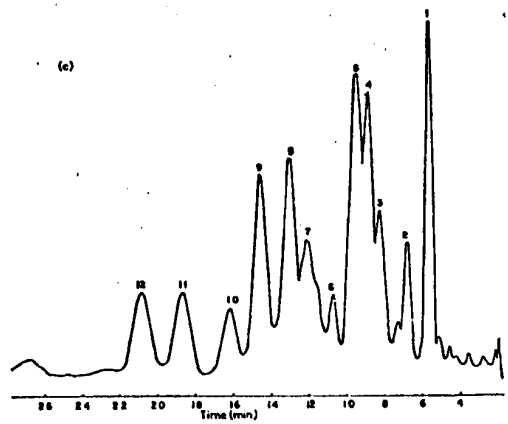

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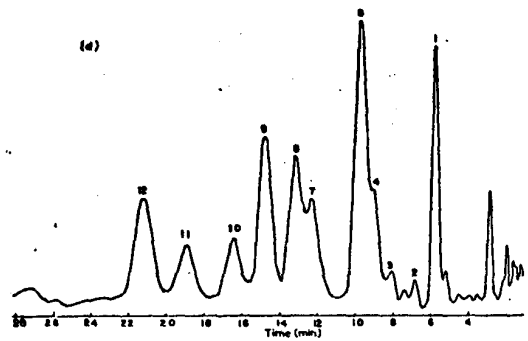

Fig. 1. *(continued)*.

Relative peak heights and chromatograms for the tissue extract of birds given Aroclor as a single capsule dose were similar to those of the Aroclor standard after partitioning (Table 1 and Figs. 1a and c). This suggests that all components of the Aroclor were readily absorbed. Relative peak heights in chromatograms for birds sacrificed 14 (day 28) and 42 (day 56) days post-treatment (Figs. 1e and f) show a methodical elimination of certain components and an increase in others by comparison with the chromatograms for the birds sacrificed after 14 days' dosage (Fig. 1d). After 42 days of untreated food, only four major components remained, peaks Nos. 5, 9, 10, and 12. Peaks 10 and 12 increased significantly. Peaks beyond 12 showed no significant change.

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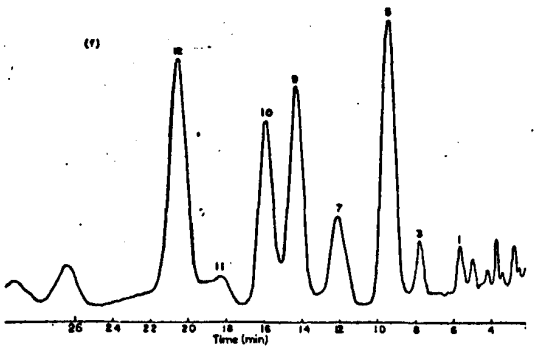

Fig. 1. *(continued).*

The chromatograms show that a dynamic system is at work, slowly removing certain components while increasing others. MS data confirmed all peaks observed in tissue samples to be identical to the ones in the Aroclor 1254 standard. No foreign chlorinated isomers were detected.

Isomerization of PCB

Isomeric transformation of PCB in natural or biological systems has not been reported. The evidence presented here strongly indicates that isomerization is taking place. Peaks Nos. 10 and 12 containing 6 chlorines show a distinct increase while peak No. 8, also containing 6 chlorines, shows a gradual decrease.

Quantitation of individual peaks was not attempted; however, when an identical amount of extract for each sample group was injected into the gas chromatograph, the distinct pattern of disappearance of some peaks and increase or decrease of others was as apparent as in Table I. Thus the changes cannot be explained simply by faster elimination of certain components.

GRANT *et al.*[14], in their study of the metabolism of Aroclor 1254 in male rats, found that the GLC-electron capture pattern of the residues was different from the standard. They concluded that all components were not metabolized at the same rate.

Peak No. 3 in the Aroclor standard is composed of a tetrachloro- and pentachlorobiphenyl, the latter in much greater proportion. A similar peak composition is observed for the capsule-dosed birds, and the birds sacrificed after 14 days of treatment. By 14 days post-treatment dechlorination apparently had occurred. After 42 days the peak had increased and was essentially the tetrachlorophenyl. Dechlorination of the pentachloro component is probable, for if the pentachloro was eliminated, the peak height should decrease significantly.

TIC chromatograms of the liver samples from the birds sacrificed after 14 days' treatment were similar to the Aroclor 1254 partitioned standard. At 42 days post-treatment the components of Aroclor 1254 were hardly in evidence; however, several

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large peaks (out of the linear range) were observed. Two major peaks appeared at relative retention times (R_x) of 0.44 and 0.82 (with retention time of *p,p'*-DDE equal to 1) and masked the Aroclor profile. MS analysis showed these to be butyl esters of short-chain fatty acids. It is suspected, since the temperature was 240°, that these were thermal degradation products of higher-molecular-weight lipoidal compounds. These compounds were observed only in the liver of treated birds, and might be related to the "fatty degeneration" observed by Vos AND KOEMAN[15] in the livers of PCB-dosed chickens. No significant peaks were observed in extracts of livers of control birds.

Analysis of a 100-g sample of feed showed approx. 0.02 p.p.m. of DDT. None was detected in birds.

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PREPARATIVE I
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SUMMARY

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INTRODUCTION

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