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Gas-Liquid Chromatography–Mass Spectrometry Characterization of Polychlorinated Biphenyls (Aroclors) and ^{36}Cl-Labeling of Aroclors 1248 and 1254

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The isomer composition of the Aroclor 1200 series was characterized by GLC-MS, using temperature programming and SE-30 support-coated, open-tubular capillary columns. A method is described for the preparation and purification of ^{36}Cl-labeled Aroclors 1248 and 1254. Neutron irradiation of the commercial material was used to prepare the ^{36}Cl-labeled material. Purification of the irradiated product was accomplished by silicic acid column chromatography. Yields of the purified product were between 63 and 99%, with no detectable alteration of the isomer composition; 10% of the ^{36}Cl produced was associated with the unchanged PCB isomers and the remaining radioactivity was contained in polychlorinated terphenyls. The terphenyls were produced by irradiation polymerization, and they were easily separated from the PCB components. Mass spectrometry, utilizing either temperature programmed GLC or direct probe sample introduction, was used to characterize the irradiation products. The radioactive materials are being utilized in experiments to determine uptake and metabolism of PCB by aquatic organisms.

Polychlorinated biphenyl (PCB) compounds have been found in fish and wildlife from Sweden (1), Britain (2, 3), The Netherlands (2, 3), and the United States (4) at levels which may interfere with pesticide residue analyses. PCB compounds have gas-liquid chromatographic (GLC) characteristics similar to many organochlorine pesticides and Jensen (5) first identified unknown GLC peaks as PCB in extracts of pike and an eagle analyzed for organochlorine insecticides. The environmental occurrence, uses, and present toxicological aspects of PCB were recently reviewed (6). The Monsanto Company has restricted the sale of PCB for uses in which disposal of the end products cannot be controlled, such as use of PCB as plasticisers (7).

The Monsanto Company is the sole manufacturer of PCB in the United States (7) and markets 8 formulations of chlorinated biphenyls under the trademarks Aroclor® 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The last 2 digits designate the per cent chlorine of each formulation. Aroclor 1254 is the material produced in greatest quantities and is used in closed-system heat exchangers and as a dielectric fluid in capacitors (8). Aroclor 1242 is used as a hydraulic fluid and Aroclor 1260 as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclors 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor 4465.

Several analytical procedures have been proposed for chromatographic separation of PCB and pesticides. Separation of PCB from pesticides by thin layer chromatography (9) with aluminum oxide does not separate aldrin, heptachlor, or DDE from PCB. Reynolds (10) reported that chromatography on a Florisil column removed PCB from 5 of 8 pesticides evaluated. Aldrin, heptachlor, and DDE eluted in the PCB fraction. Column chromatography with silicic acid (11) separated the various Aroclor formulations from all of the common chlorinated pesticides except aldrin. This procedure is quite satisfactory for use in routine separations of PCB and pesticides; however, the deactivation of the silicic acid with water must be done precisely.

Limited information exists on the biological consequences of PCB exposures or residues in fish. A synergistic toxicological interaction in flies was reported between PCB and the insecticides dieldrin and DDT (9). Our principal concern in preparing radioactive ^{36}Cl-labeled PCB components is to delineate their dynamics in fish and other components of aquatic ecosystems.

We describe the characterization of Aroclor formulations by programmed temperature GLC-mass spectrometry and the preparation and purification of neutron-irradiated Aroclors 1248 and

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1254. An analytical procedure was evaluated by use of purified ^{36}Cl-labeled Aroclor formulations.

Experimental

Sample Preparation

Quartz vials (3 × ⅜″ id) containing 0.5 or 1.0 g Aroclor 1254 and 1.0 g Aroclor 1248 were cooled with Dry Ice and sealed under vacuum with a torch. The vials were wrapped with aluminum foil, seal-welded in a 4 × 1½″ aluminum can, and leak-tested.

Neutron Irradiation

Preliminary irradiations of 0.5 g Aroclor 1254 were made for 60 hr and for 36 min at a neutron flux density of 4–5 × 10^{13} neutron/cu cm and for 6 hr at a flux density of 6–7 × 10^{12} neutrons/cu cm. Aroclors 1248 and 1254 (1.0 g each) were irradiated 40 min at a flux density of 4–5 × 10^{13} neutrons/cu cm. The irradiations were conducted at the Research Reactor Facility of the University of Missouri, Columbia, Mo. After irradiation, activity in the vials was allowed to decay for a minimum of 3 days prior to opening the vials.

Sample Solution

The vials were crushed with a metal rod in a 100 ml heavy-walled bottle, capped with a Teflon®-lined cap having a ¼″ hole. This and all subsequent manipulations of the material were conducted in a hood designed for radiochemical operations. The irradiated materials were dissolved in redistilled hexane. This solution was filtered through glass wool into a 25 ml volumetric flask. Twenty-five µl of the solution was diluted 100-fold and a 100 µl aliquot was used for determination of the radioactivity produced.

Radioactive Assay

A Beckman 200-L liquid scintillation counter with a variable isoset discriminator, set for the ^{36}Cl-β-particle energy spectrum, was used to determine all radioactivity. Samples were placed in glass scintillation vials containing 10.0 ml Beckman Fluoralloy® dry mix dissolved in toluene. The fluor contained 8.0 g/L butyl PBD (2-(4′-*tert.*-butylphenyl)-5-(4′-biphenyl)-1,3,4-oxidozole) and 0.5 g/L PBBD (2-(4′-biphenyl)-6-phenyl-benzolasole). Counting efficiency for ^{36}Cl was determined to be 98%.

Silicic Acid Cleanup

The irradiated Aroclor formulations were concentrated to <5 ml and pipetted onto a glass column 2.2 cm id × 50 cm long containing 25 g silicic acid-Celite mixture. Silicic acid-Celite 545 column chromatography is described by Armour and Burke (11); however, we modified the column preparation method for cleanup of our samples. The silicic acid, Celite 545, and water (equivalent to 3% of silicic acid added) were weighed or measured into a large-mouth 1 gal. glass jar capped with a hexane-rinsed, aluminum-lined cap and tumbled for 4–5 hr. The pre-mixed column material (ca 500 g) was then covered with redistilled petroleum ether and slurried before preparation of the chromatographic column. The slurried silicic acid-Celite was poured into a glass column (equipped with a Teflon stopcock) to a height equivalent to 25 g adsorbent. The sample was added and 250 ml petroleum ether under slight pressure was percolated through the column at 4–5 ml/min. The first 200 ml of petroleum ether was collected and designated as fraction I-A. The last 50 ml of the petroleum ether eluate was discarded because of the possibility of contamination by terphenyl materials. The column was then eluted with 200 ml acetonitrile-hexane-methylene chloride (1 + 19 + 80, v/v/v); this eluate was designated as fraction II and was examined by GLC-MS and MS with the direct probe. After this initial cleanup, the PCB components in fraction I-A of the irradiated 1.0 g samples of Aroclors 1248 and 1254 were evaporated separately to <5 ml and pipetted onto and eluted from individual silicic acid-Celite columns with 200 ml petroleum ether. These eluates were designated fraction I.

Sample Characterization

A gas chromatograph-mass spectrometer (GLC-MS) was used to determine the composition of the eluted materials from each Aroclor formulation. The PCB components in fraction I from each irradiated Aroclor were chromatographed, using a 50′ × 0.020″ id SE-30 support-coated, open-tubular (SCOT) capillary column. Gas chromatograms were also obtained from standard solutions of Aroclors 1248 and 1254. The materials in fraction II from each irradiated Aroclor were examined by mass spectrometry, introducing the sample with the direct probe. The mass spectra were also determined for Aroclors 1248, 1254, 5442, and 5460 with the direct probe.

The irradiated materials prior to cleanup were spotted on 2 × 8″ strips of Eastman Chromatogram sheet (Type K-301-R, Distillation Production Industries, Rochester, N.Y.) and developed in hexane. A Packard Model 7201 radio-chromatogram scanner (Packard Instrument Co., Inc., Downers Grove, Ill.) was used for detection, measurement, and recording of the radioactivity in the components separated by thin layer chromatography.

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Mass Spectrometry

Mass spectrometric analyses were performed with a double focusing, low resolution mass spectrometer (Model 270, Perkin-Elmer Corp., Norwalk, Conn.) coupled through a Watson-Biemann molecular separator to a temperature programmed gas chromatograph. The GLC column was a 50′ × 0.020″ SE-30 SCOT chromatographic column. Helium at 9 psig was used as the carrier gas. The temperature program rate was 2°C/min. In most cases, the initial temperature was 150°C with a 2 min delay prior to temperature programming. The molecular separator was maintained at 260°C and the GLC inlet was held at 220°C. The ion source and target were maintained at 150 and 130°C, respectively. All mass spectra were recorded at 70 eV electron energy with 2000 v accelerating potential. The filament emission current was 100 μa. Samples introduced by the direct probe were introduced at a probe temperature of ca 70°C and, after introduction, were heated to 250°C at ca 10°C/min. Spectra scan rates were 3 sec/decade from 20 to 800 atomic mass units. Perfluordecalin was used as a mass calibration standard (12).

Results and Discussion

GLC-MS Characterization of the Aroclor Series

The composition of the various Aroclor formulations is best illustrated by Figs. 1 and 2. The numbers above the gas chromatographic peaks represent the number of chlorine atoms substituted on the biphenyl molecule. Chromatographic peaks designated as containing a PCB isomer with a given number of chlorine atoms are the same as undesignated peaks immediately above or below a numbered peak. These values were determined from the mass spectrum of each chromatographic peak. The molecular weights of the possible compounds composing the Aroclor formulations are listed in Table 1.

Each Aroclor formulation contained a group of predominant, characteristic isomers. The composition of the most predominant isomers (Figs. 1 and 2) and the per cent chlorine in each isomer (Table 1) is related to the per cent chlorine in each Aroclor formulation. For example, Aroclor 1221 contains 20% chlorine and its predominant isomer, chlorobiphenyl, is 18.6% chlorine; some dichlorobiphenyl isomers (31.5% chlorine) are also present. Aroclor 1248 contains tri, tetra-, and pentachlorobiphenyl isomers, which contain 41.02, 48.28, and 54.01% chlorine, respectively. Aroclor 1254 contains only trace levels of trichloro isomers. Similarly, Aroclor 1268 has

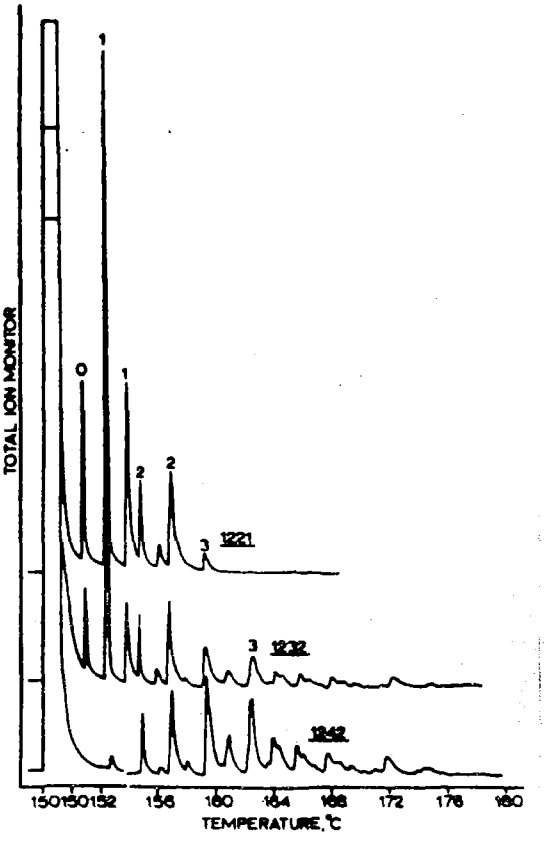

FIG. 1—GLC-MS separation of Aroclor materials. Underlined numbers designate the Aroclor formulation and numbers above the peaks designate the number of chlorine atoms in the compound(s) determined from the mass spectrum. Temperature program rate 2°C/min, He 9 psig, 50′ × 0.020″ SE-30 SCOT column, PE-270 GLC-MS.

principally octa- and nonachlorobiphenyl isomers, which are 65.73 and 68.47% chlorine, respectively.

The mass spectra of the chlorinated biphenyl and terphenyls are characterized by a strong parent ion peak with an isotope distribution determined by the number of chlorine atoms substituted on the aromatic rings. The spectra are quite simple and are readily identified. Bagley *et al.* (13) have reported the mass spectra of the components in Aroclor 1254.

The mass spectra representing Aroclors 5442 and 5460 indicate that Aroclors 5442 and 5460 are composed of chlorinated terphenyls substituted with no more than 6 or 11 chlorine atoms, respectively. However, traces of isomers containing 7 or 12 chlorine atoms were detected. These

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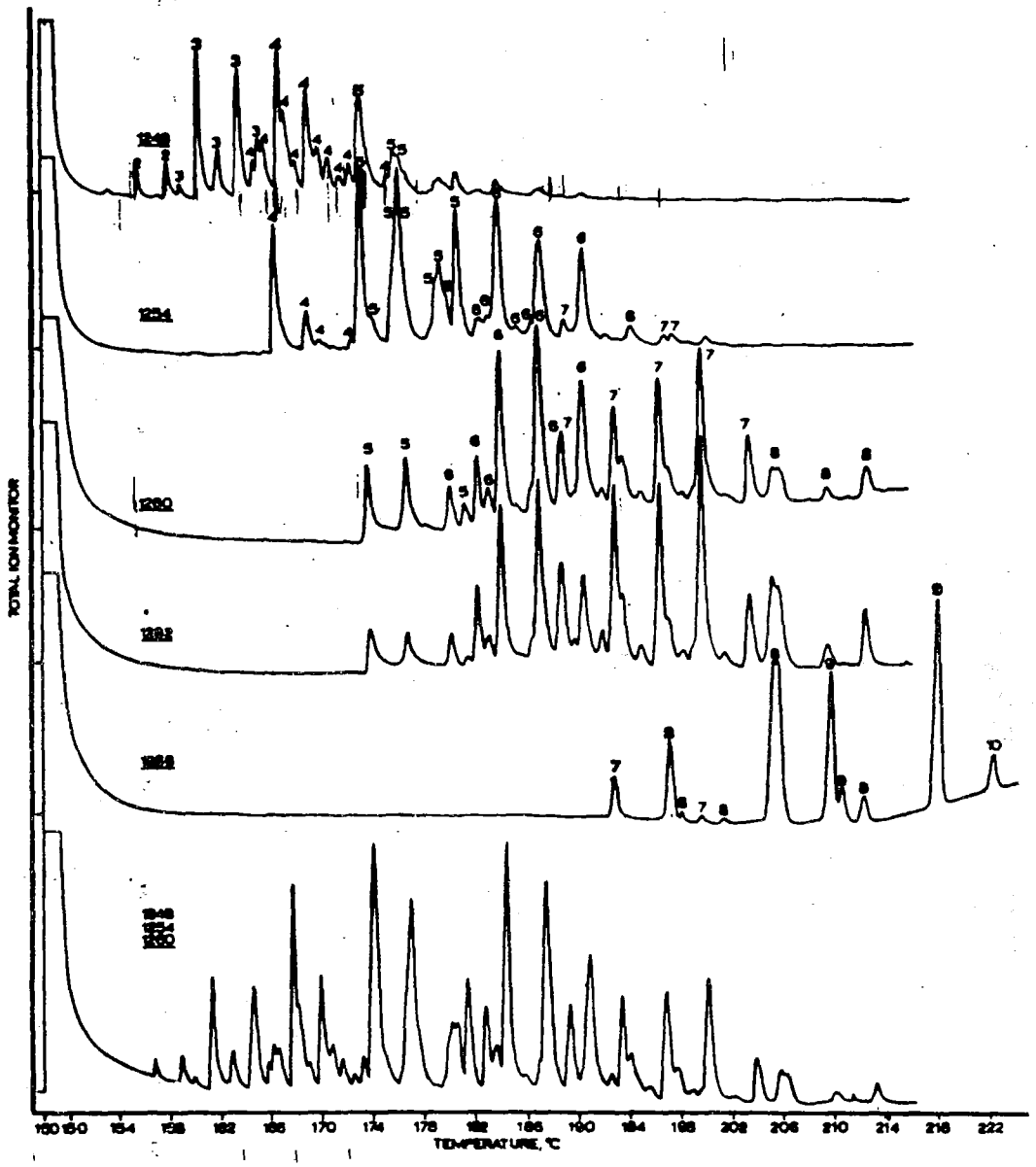

FIG. 2—GLC-MS separation of Aroclor materials. See Fig. 1 for explanation and experimental conditions.

isomers contain 48.39% (6 chlorine atoms) and 63.74% (11 chlorine atoms) chlorine, respectively (Table 1). Further isomer characterization of the chlorinated terphenyl Aroclor formulations must await the development of more suitable GLC-MS chromatographic conditions.

^{36}Cl-Labeled Aroclors 1248 and 1254

Chlorine-36 is produced by an (n, γ) reaction with ^{35}Cl. The cross-section for neutron capture by ^{35}Cl is 45 barns. The half-life of ^{36}Cl is 5000 yr and this isotope emits a 0.7 MeV β particle. Chlorine-38 is also produced from ^{37}Cl by an (n, γ) reaction, but its half-life is only 37 min and the cross-section for the (n, γ) reaction is 0.56 barns.

Thin layer chromatography and temperature programmed GLC combined with mass spectrometry are suitable for determining the compositional changes which occur in Aroclor materials as a result of neutron irradiation. The Aroclor

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Table 1. Empirical formula, molecular weights, and per cent chlorine of chlorinated biphenyls and terphenyls (all calculations are based on ^{35}Cl)

Chlorinated Biphenyls			Chlorinated Terphenyls		
Empirical Formula	Mol. Wt	% Cl	Empirical Formula	Mol. Wt	% Cl
$C_{12}H_{10}$	154	0.00	$C_{18}H_{14}$	230	0.00
$C_{12}H_9Cl$	188	18.62	$C_{18}H_{13}Cl$	264	13.26
$C_{12}H_8Cl_2$	222	31.53	$C_{18}H_{12}Cl_2$	298	23.49
$C_{12}H_7Cl_3$	256	41.82	$C_{18}H_{11}Cl_3$	332	31.63
$C_{12}H_6Cl_4$	290	48.28	$C_{18}H_{10}Cl_4$	366	38.25
$C_{12}H_5Cl_5$	324	54.61	$C_{18}H_9Cl_5$	400	43.75
$C_{12}H_4Cl_6$	358	59.66	$C_{18}H_8Cl_6$	434	48.39
$C_{12}H_3Cl_7$	392	62.50	$C_{18}H_7Cl_7$	468	52.35
$C_{12}H_2Cl_8$	426	65.73	$C_{18}H_6Cl_8$	502	55.78
$C_{12}H_1Cl_9$	460	68.47	$C_{18}H_5Cl_9$	536	58.78
$C_{12}H_6Cl_{10}$	494	79.85	$C_{18}H_4Cl_{10}$	570	61.49
			$C_{18}H_3Cl_{11}$	604	63.74
			$C_{18}H_2Cl_{12}$	638	65.83
			$C_{18}H_1Cl_{13}$	672	67.71
			$C_{18}H_6Cl_{14}$	706	69.41

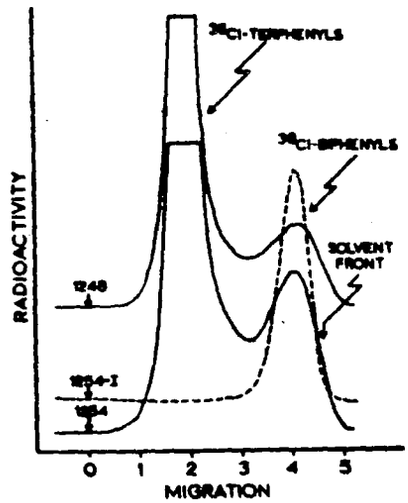

FIG. 3—Radiochromatograms from thin layer separations of neutron-irradiated Aroclor formulations: —, irradiated Aroclors 1248 and 1254; - - - -, radioactive PCB material eluting in fraction I from silicic acid cleanup of irradiated material.

1254 irradiated for 60 hr was not usable because the material decomposed and was insoluble in hexane. The radiochromatograms of Aroclors 1248 and 1254 irradiated 40 min revealed the presence of 2 zones of radioactive material with R_f values of 0.90 and 0.47 (Fig. 3). No difference in R_f values of the radioactive PCB compounds was observed for Aroclors 1248 and 1254 (R_f value = 0.90). Radioactivity of the slower migrating material was approximately 10 times that of the radioactive PCB. Silicic acid column chromatography with petroleum ether (fraction I) eluted only the radioactive PCB materials (R_f 0.90).

The mixture of hexane-acetonitrile-methylene chloride eluted the slower migrating material (R_f 0.47) in fraction II. The radioactivity associated with the column eluates from various irradiations is presented in Table 2. Comparison of GLC-MS chromatograms of Aroclors 1248 and 1254 with fraction I of the irradiated materials revealed no significant alteration of the concentration ratios of individual isomers of either PCB material (Figs. 4 and 5).

The specific activity of PCB recovered in fraction I of the 6 hr irradiated Aroclor 1254 was greater than that of PCB exposed 36 min at higher neutron flux and eluted in fraction I. The composition of PCB exposed to the higher neutron flux was similar to the starting material except that the relative concentrations of 2 major isomers (each containing 5 chlorine atoms) were increased by 10–15%. The specific activity of the irradiated Aroclors 1248 and 1254 recovered in fraction I (Table 2) was expected to be slightly higher for Aroclor 1254 than for Aroclor 1248 because more chlorine/g was irradiated in Aroclor 1254.

The mass spectra of the material eluted in fraction II from the irradiated Aroclor 1248 had molecular ions and ion fragment peaks which were identical to those of Aroclor 5460. However, the relative intensity of the spectral fragments of the various chlorinated terphenyl compounds is greater for the higher ions representing chlorinated isomers in the irradiated materials. The spectra of the irradiated Aroclor 1254 in fraction II were composed of isomers of terphenyl from the pentachloroterphenyls to the fully chlorinated ($C_{18}Cl_{14}$) terphenyl ion of 706 atomic mass units.

Gas chromatographic peaks were not observed for Aroclors 5442 and 5460 under the conditions employed in the GLC-MS separation of the chlorinated biphenyl compounds. Polychlorinated terphenyls are produced during the irradiation exposure. These materials may result from free radical reactions of chlorinated phenyl radicals with chlorinated biphenyl molecules. These free radicals could be produced by the recoil energy of (n, γ) reactions of chlorine in the chlorinated biphenyl molecules. The radioactive chlorinated terphenyls are separated from the chlorinated biphenyl compounds by the silicic acid column. In

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Table 2. Radioactivity produced by neutron irradiation of Aroclors 1248 and 1254

Aroclor		Neutron		Radioactivity		Recovery, mg	Specific Activity, dpm/μg
Formulation	Sample, g	Flux, neutrons/cu cm	Exposure	Fraction	dpm		
1248	1.0	$4–5 \times 10^{13}$	40 min	initial[a]	1.44×10^{9}	995	69.5
				I	1.63×10^{8}		
				II	1.23×10^{9}		
				total	1.40×10^{9}		
1254	0.5	$6–7 \times 10^{12}$	6 hr	initial	2.9×10^{9}		
				I-A	4.7×10^{7}	265	177
1254	0.5	$4–5 \times 10^{13}$	36 min	initial	1.25×10^{9}		
				I-A	2.69×10^{7}	425	53
1254	1.0	$4–5 \times 10^{13}$	40 min	initial	1.53×10^{9}		
				I	1.62×10^{8}	975	90.2
				II	1.3×10^{9}		
				total	1.48×10^{9}		

[a] Activity prior to silicic acid cleanup.

our experiments, the radioactive chlorinated terphenyls were eluted by the more polar solvent system: hexane-acetonitrile-methylene chloride. However, Armour and Burke (11) reported that chlorinated terphenyl Aroclor materials were not recovered in the pesticide fraction but were eluted with 250 ml petroleum ether.

Radioactive and non-radioactive Aroclors and non-radioactive *p,p'*-isomers of DDE, DDD, and DDT were used to evaluate the separation of PCB and pesticides with this silicic acid column chromatographic technique. Seven per cent of the *p,p'*-DDE eluted in fraction I along with PCB (Table 3). The amount of Aroclors eluted in the pesticide fraction was also determined. The elution volumes required to achieve the optimum separation of PCB and pesticides (225 ml) should be determined for each preparation of silicic acid-Celite 545.

Conclusions

Temperature programmed GLC with SCOT columns should be seriously considered for use to determine the relative uptake in biological studies of the various isomers in Aroclor formulations. We feel that residues of PCB compounds composed of mixtures of Aroclor formulations can be analyzed by this technique. Each mixture is characterized by a particular isomer pattern and contains isomers not present in the Aroclor formulations of next higher or lower number. A series of linear equations is required to determine the relative amounts of each Aroclor formulation present. However, in biological or sediment sam-

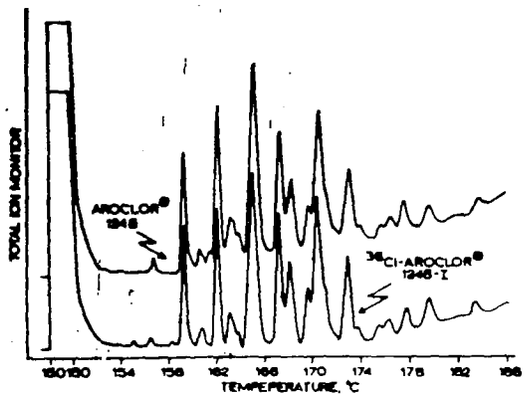

FIG. 4—SCOT GLC-MS comparison of Aroclor 1248 and radioactive Aroclor 1248 material eluted in fraction I of silicic acid cleanup. See Fig. 1 for experimental conditions.

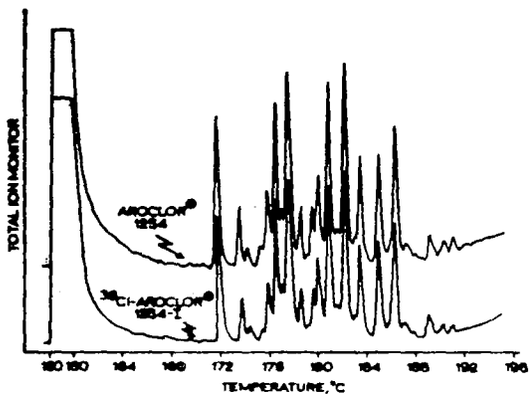

FIG. 5—SCOT GLC-MS comparison of Aroclor 1254 and radioactive Aroclor 1254 material eluted in fraction I of silicic acid cleanup. See Fig. 1 for experimental conditions.

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Table 3. PCB and pesticide recoveries with silicic acid chromatography

Fraction	Solvent	Volume, ml	% Recovery: Aroclor 1248[a]	Aroclor (^{36}Cl)[b]	Aroclor 1254	Aroclor (^{36}Cl)	Pesticide: DDE[c]	Pesticide: DDD	Pesticide: DDT
PCB	petroleum ether	0–200	95	95.7	95	97.6	0	—	—
		200–225	4		4		7	—	—
		225–250	1	2.7[d]	1	1.1[d]	34	—	—
			100	98.4	100	98.7	41	0	0
Pesticide	C_6H_{14}—CH_3CN—CH_2Cl_2	0–200	trace	1.6	trace	1.3	59	100	100
Total, %			100	100	100	100	100	100	100
Total, μg			5	60	5	51	5	5	5

[a] Recoveries determined by GLC.
[b] Recoveries determined using ^{36}Cl-radiolabeled material.
[c] *p,p'*-isomers of DDE, DDD, and DDT.
[d] Total in 200–250 ml eluate.

ples, these calculations would require knowledge of changes produced in the Aroclor composition, an unknown factor at the present time.

Radioactive ^{36}Cl-labeled Aroclors 1248 and 1254 can be prepared by neutron irradiation. Specific activities of 75–100 disintegrations per min (dpm)/μg were obtained by neutron irradiation at flux densities of 4–5 × 10^{13} neutrons/cu cm. The irradiated materials exhibited no detectable changes in isomer composition when silicic acid-Celite 545 column chromatography was employed for purification. We assume that the radioactivity of the various PCB isomers is distributed in the same manner as the probability of neutron capture.

The utility of using radioactive polychlorinated biphenyls in analytical methodology has been demonstrated by the evaluation of the procedure for chromatographic separation of PCB and pesticides with silicic acid column chromatography. Only 1.6% Aroclor 1248 and 1.3% Aroclor 1254 remained in the pesticide fraction and 7% of the *p,p'*-DDE eluted in the PCB fraction. The greatest benefit of these radioactive materials will become apparent when results of biological uptake and metabolism studies with aquatic organisms are available.

Note added in proof.—Since the preparation of this manuscript, we have detected the presence of ^{35}S-labeled PCB components in the purified ^{36}Cl-labeled PCBs. Sulfur-35 is produced by (n,p) reaction of ^{35}Cl; however the cross-section for the (n,p) reaction is 0.30 barns as compared to 45 barns for the (n,γ) reaction to produce ^{36}Cl. The ^{35}S-components have not been chemically characterized. Except for a shorter half life, the thin layer and silicic acid chromatographic properties and partition values of the ^{35}S-PCB components do not appear different from those of the ^{36}Cl materials. Additional research to elucidate the distribution of the radioactivity among the various PCB components is required.

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