GC-MS Characterization of PCBs (Aroclors) and ^{36}Cl Labeling of Aroclor® 1248 and 1254

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The isomer composition of the Aroclor 1200 series was characterized by GC-MS using temperature programming and SE-30 SCOT capillary columns. A method is described for the preparation and purification of ^{36}Cl-labeled Aroclor® 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 percent, with no detectable alteration of the isomer composition. Ten percent of the ^{36}Cl produced was associated with the unchanged PCB isomers. The remaining radioactivity was contained in polychlorinated terphenyls. The terphenyls were produced by irradiation polymerization, and they were easily separated from the PCBs. Mass spectrometry, utilizing either temperature-programmed gas chromatography 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 PCBs by aquatic organisms.

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

The Monsanto Company is the sole manufacturer of PCBs in the United States (7) and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor® 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The latter two digits designate the percent 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 (9). Aroclor® 1242 is used as a hydraulic fluid and Aroclor® 1260 as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor® 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor® 4465.

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Several analytical procedures have been proposed for chromatographic separation of PCBs and pesticides. Separation of PCBs from pesticides by thin layer chromatography (8) with Al_2O_3 does not separate aldrin, heptachlor or DDE from the PCBs. Reynolds (10) reported that chromatography on a Florisil column removed PCBs from five of eight 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 PCBs and pesticides, however, 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 PCBs and the insecticides dieldrin and DDT (8). Our principal concern in preparing radioactive ^{36}Cl-labeled PCBs is to delineate their dynamics in fish and other components of aquatic ecosystems.

We describe the characterization of Aroclor formulations by programmed temperature GLC-MS SCOT capillary column and the preparation and purification of neutron irradiated Aroclor® 1248 and 1254. An analytical procedure was evaluated by use of purified ^{36}Cl-labeled Aroclor formulations.

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EXPERIMENTAL

Sample Preparation

Quartz vials (3" x 3/8" i.d.) containing 0.5 or 1.0 g of Aroclor® 1254 and 1.0 g of Aroclor® 1248 were cooled with dry ice and sealed under vacuum with a torch. The vials were wrapped with aluminum foil and seal-welded in a 4" x 1 1/8" aluminum can and leak tested.

Neutron-irradiation

Preliminary irradiations of 0.5 g of Aroclor® 1254 were made for 60 hrs and for 36 min at a neutron flux density of 4-5 x 10^{13} n/cm^3 and for 6 hrs at a flux density of 5-7 x 10^{12} n/cm^3. Irradiation of 1.0 g of Aroclor® 1248 and 1254 was made for 40 min at a flux density of 4-5 x 10^{13} n/cm^3. The irradiations were conducted at the Research Reactor Facility of the University of Missouri, Columbia, Missouri. After irradiation, activity in the vials was allowed to decay for a minimum of 3 days prior to opening the vial.

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 1/4" 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 microliters of the solution was diluted 100-fold and a 100-µl aliquot used for determination of the radioactivity produced.

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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 of Beckman Fluoralloy® dry mix dissolved in toluene. The fluor contained 8.0 g/l butyl PBD [2-(4'-t-butylphenyl) 5-(4'-biphenyl)-1,3,4-oxidozole] and 0.5 g/l PBBD [2-(4'-biphenyl)-6-phenyl-benzolazole]. Counting efficiency for ^{36}Cl was determined to be 98 percent.

Silicic acid cleanup

The irradiated Aroclor® formulations were concentrated to less than 5 ml and pipetted onto a 2.2 cm i.d. x 50 cm long glass column containing 25 g of 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 percent 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 prior to 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 of adsorbent. The sample was applied and petroleum ether (250 ml) under slight pressure was percolated through the column at a flow rate of 4-5 ml/min.

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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 due to the possibility of contamination by terphenyl materials. The column was then eluted with 200 ml of acetonitrile-hexane-methylene chloride 1:19:80 (v:v:v); this eluate was designated as fraction II and was examined by GC-MS and MS using the direct probe. After this initial cleanup, the PCBs in fraction I-A of the irradiated 1.0-g samples of Aroclor® 1248 and 1254 were evaporated separately to less than 5 ml and pipetted onto, and eluted from individual silicic acid-celite columns with 200 ml of petroleum ether. These eluates were designated fraction I.

Sample Characterization

A gas chromatograph-mass spectrometer (GC-MS) was used to determine the composition of the eluted materials from each Aroclor formulation. The PCBs in fraction I from each irradiated Aroclor were chromatographed using a 50' x 0.020" i.d. SE-30 support-coated, open-tubular (SCOT) capillary column. Gas chromatograms were also obtained from standard solutions of Aroclor® 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 Aroclor® 1248, 1254, 5442 and 5460 with the direct probe.

The irradiated materials prior to cleanup were spotted on Eastman Chromatogram Sheet, 2" x 8" strips (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.) was used

for detection, measurement, and recording of the radioactivity in the components separated by TLC.

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-Bieman molecular separator to a temperature-programmed gas chromatograph. The GC column was a 50' x 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 GC-inlet was held at 220° C. The ion source and target were maintained at 150° C and 130° C, respectively. All mass spectra were recorded at 70 eV electron energy with 2,000 volts 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 approximately 10° C/min. Spectra scan rates were 3 sec/decade from 20 to 800 amu. Perfluordecalin was used as a mass calibration standard (12).

RESULTS AND DISCUSSION

GC-MS Characterization of the Aroclor series

The composition of the various Aroclor formulations is best illustrated by Figures 1 and 2. The number above the gas chromatographic

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peaks represents 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 (Figure 1 and 2), and the percent chlorine in each isomer (Table 1) is related to the percent chlorine in each Aroclor formulation. For example, Aroclor® 1221 contains 20-percent chlorine and its predominant isomer, chlorobiphenyl, is 18.6-percent chlorine; some dichlorobiphenyl isomers (31.5 percent chlorine) are also present. Aroclor® 1248 contains tri-, tetra- and pentachlorobiphenyl isomers which contain 41.02-, 48.28-, and 54.01-percant chlorine, respectively. Aroclor® 1254 contains only trace levels of trichloro isomers. Similarly, Aroclor® 1268 has principally octa- and nonachlorobiphenyl isomers which are 65.73- and 68.47-percent 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.

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The mass spectra representing Aroclor® 5442 and 5460 indicate that Aroclor® 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 isomers contain 48.39 (6 chlorine atoms) and 63.74 (11 chlorine atoms) percent chlorine, respectively (Table 1). Further isomer characterization of the chlorinated terphenyl Aroclor formulations must await the development of more suitable GC-MS chromatographic conditions.

^{36}Cl-labeled Aroclor® 1248 and 1254

Chlorine-36 is produced by a (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 3,000 yrs and this isotope emits a 0.7 mev β^- particle. Chlorine-38 is also produced from ^{37}Cl by a (n,γ) reaction, but its half-life is only 37 minutes and the cross section for the (n,γ) reaction is 0.56 barns.

Thin layer chromatography, and temperature-programmed gas chromatography combined with mass spectrometry are suitable for determining the compositional changes which occur in Aroclor materials as a result of neutron irradiation. The Aroclor® 1254 irradiated for 60 hrs was not usable because the material decomposed and was insoluble in hexane. The radiochromatograms of Aroclor® 1248 and 1254 irradiated for 40 min revealed the presence of two zones of radioactive material with R_f values of 0.90 and 0.47 (Figure 3). No difference in R_f values of the radioactive PCBs were observed for Aroclor® 1248 and 1254 and the R_f values were 0.90. Radioactivity of the slower migrating material

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was approximately 10 times that of the radioactive PCBs. 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 GC-MS chromatograms of Aroclor® 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 (Figures 4 and 5).

The specific activity of the PCBs recovered in fraction I of the 6-hr irradiated Aroclor® 1254 was greater than that of PCBs exposed for 36 min at higher neutron flux and eluted in fraction I. The compositition of the PCBs exposed to the higher neutron flux was similar to the starting material except that the relative concentrations of two major isomers (each containing 5 chlorine atoms) were increased by 10-15 percent. The specific activity of the irradiated Aroclor® 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 was irradiated per gram of 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

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in the irradiated materials. The spectra of the irradiated Aroclor® 1254 in fraction II was composed of isomers of terphenyl from the pentachloroterphenyls to the fully chlorinated ($C_{18}Cl_{14}$) terphenyl ion of 706 amu.

Gas chromatographic peaks were not observed for Aroclor® 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 our experiments, the radioactive chlorinated terphenyls were eluted by the more polar solvent system; hexane-acetonitrile-methylene chloride. However, Armour and Burke (11) reported 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 PCBs and pesticides with this silicic acid column chromatography technique. Seven percent of the p,p'-DDE eluted in fraction I along with the PCBs (Table 3). The amount of Aroclors eluted in the pesticide fraction was also determined. The elution volumes required to achieve the optimum separation of PCBs and pesticides (225 ml) should be determined for each preparation of silicic acid-Celite 545.

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CONCLUSIONS

Temperature-programmed gas chromatography 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 PCBs 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 samples these calculations would require knowledge of changes produced in the Aroclor composition, an unknown factor at the present time.

Radioactive ^{36}Cl labeled Aroclor® 1248 and 1254 can be prepared by neutron irradiation. Specific activities of 75-100 dpm/μg were obtained by neutron irradiation at flux densities of 4-5 x 10^{13} n/cm^3. The irradiated materials exhibited no detectable changes in isomer compos-tion when silicic acid-Celite 545 column chromatography is 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 PCBs and pesticides with silicic acid column chromatography. Only 1.6-percent Aroclor® 1248 and 1.3-percent Aroclor® 1254 remained in the pesticide fraction and 7 percent 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 completed.

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Table 1. Emperical formula, molecular weights and percent chlorine of chlorinated biphenyls and terphenyls. All calculations based on ^{35}Cl.

Chlorinated Biphenyls			Chlorinated Terphenyls		
Emperical formula	MW	%Cl	Emperical formula	MW	%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	41.02	$C_{18}H_{11}Cl_3$	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	54.01	$C_{18}H_9Cl_5$	400	43.75
$C_{12}H_4Cl_6$	358	58.66	$C_{18}H_8Cl_6$	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
$C_{12}H_0Cl_{10}$	494	79.85	$C_{18}H_4Cl_{10}$	570	61.40
			$C_{18}H_3Cl_{11}$	604	63.74
			$C_{18}H_2Cl_{12}$	638	65.83
			$C_{18}H_1Cl_{13}$	672	67.71
			$C_{18}H_0Cl_{14}$	706	69.41

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

Aroclor® Formulation	Aroclor® sample (g)	Neutron Flux, n/cm^2	Neutron Exposure	Radioactivity fraction	Radioactivity dpm	Recovery (mg)	Specific activity (dpm/μg)
1248	1.0	4-5x10^{13}	40 min	initial[1]	1.44x10^9	995	69.5
				I	4.63x10^8		
				II	1.23x10^9		
				Total	1.40x10^9		
1254	0.5	6-7x10^{12}	6 hrs	initial	2.9x10^9		
				I-A	4.7x10^7	265	177
1254	0.5	4-5x10^{13}	36 min	initial	1.25x10^9		
				I-A	2.69x10^7	425	53
1254	1.0	4-5x10^{13}	40 min	initial	1.53x10^9		
				I	1.62x10^8	975	90.2
				II	1.3x10^9		
				Total	1.48x10^9		

[1] Activity prior to silicic acid cleanup

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Table 3. PCB and pesticide recoveries with silicic acid chromatography.

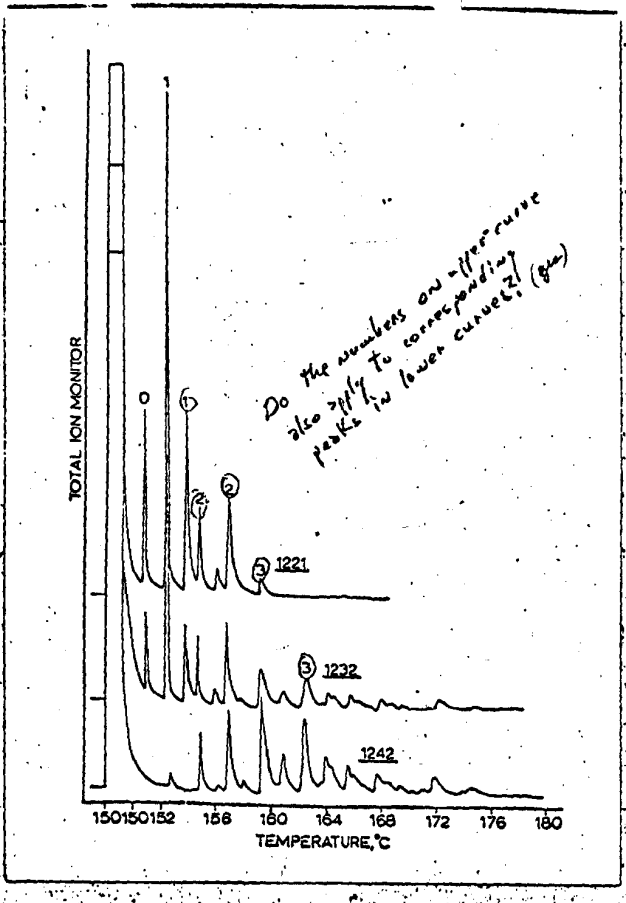
Fraction	Solvent	Volume (ml)	% Recovery: Aroclor® 1248[1]	Aroclor® (^{36}Cl)[2]	Aroclor® 1254	Aroclor® (^{36}Cl)	Pesticide: DDE[3]	Pesticide: DDD	Pesticide: DDT
PCB	Petroleum Ether	0-200	95	95.7	95	97.6	0	-	-
		200-225	4	2.7[4]	4	1.1[4]	7	-	-
		225-250	1		1		34	-	-
			100	98.4	100	98.7	41	0	0
Pesticide	C_6H_{14}-CH_3CN-CH_2Cl_2	0-200	tr	1.6	tr	1.3	59	100	100
		total, %	100	100	100	100	100	100	100
		total, µg	5	60	5	51	5	5	5

1 Recoveries determined by gas liquid chromatography
2 Recovery determined using ^{36}Cl-radiolabeled material
3 p,p-isomers of DDE, DDD and DDT
4 Total in 200-250 ml eluate

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Figure 1. GC-MS of Aroclor® PCBs. SCOT GC-MS separation of Aroclor materials. The 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' x 0.020" SE-30 SCOT column, PE-270 GC-MS.

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Do the numbers on the upper curve also apply to corresponding peaks in lower curve? (Yes)

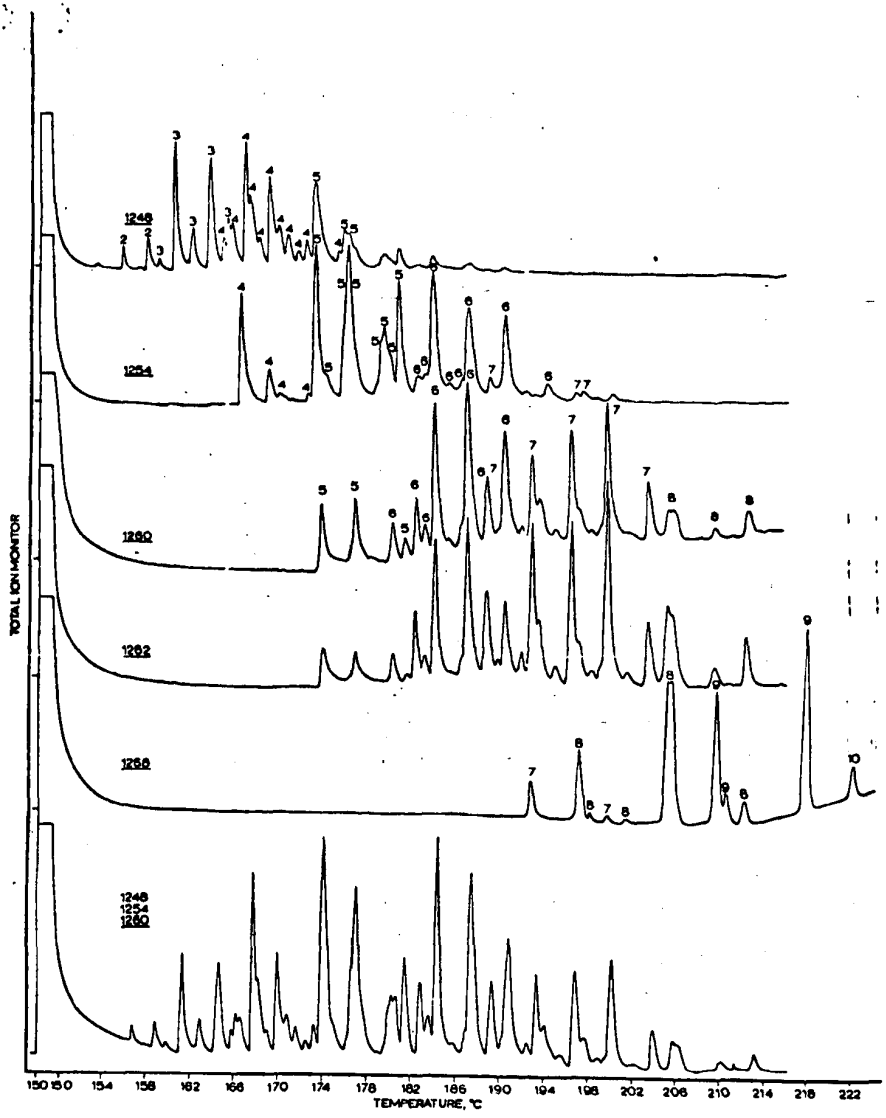
Fig. 1

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Figure 2. GC-MS of Aroclor® PCBs. SCOT GC-MS separation of Aroclor materials. The 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' x 0.020" SE-30 SCOT column, PE-270 GC-MS.

Figure 3. Radiochromatograms from thin layer separations of neutron irradiated Aroclor formulations. Solid curves represent irradiated Aroclor® 1248 and 1254. Dashed curve represents the radioactive PCB material eluting in fraction I from the silicic acid cleanup of the irradiated material.


1248
1254
1260
1262
1268
1248
1254
1260
TOTAL ION MONITOR
150 150 154 158 162 166 170 174 178 182 186 190 194 198 202 206 210 214 218 222
TEMPERATURE, °C

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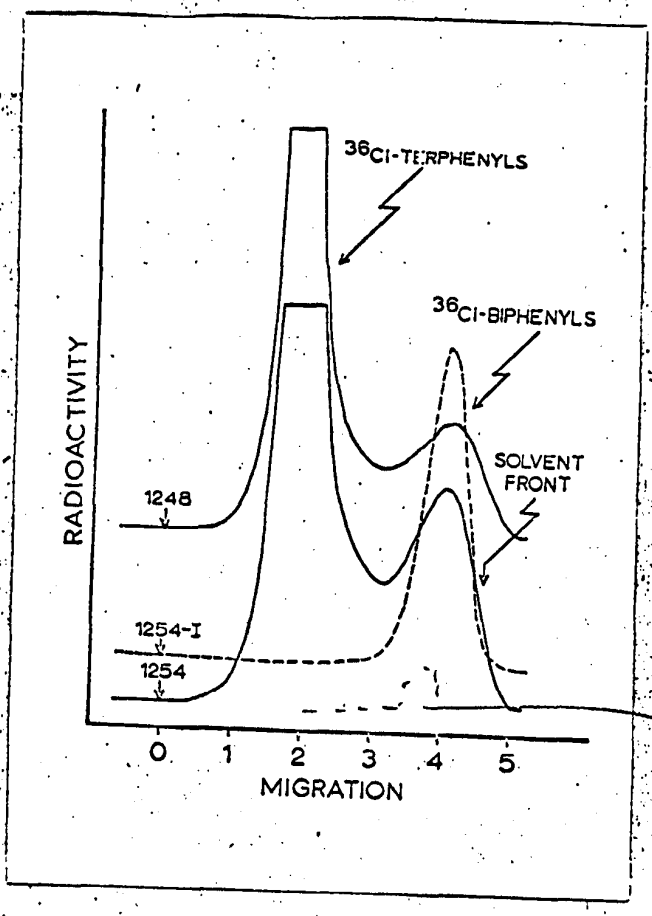


Fig. 3

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Figure 4. SCOT GC-MS comparison of Aroclor® 1248 and the radioactive Aroclor® 1248 material eluted in fraction I of the silicic acid cleanup. Chromatographic conditions are as described in Figure 1.

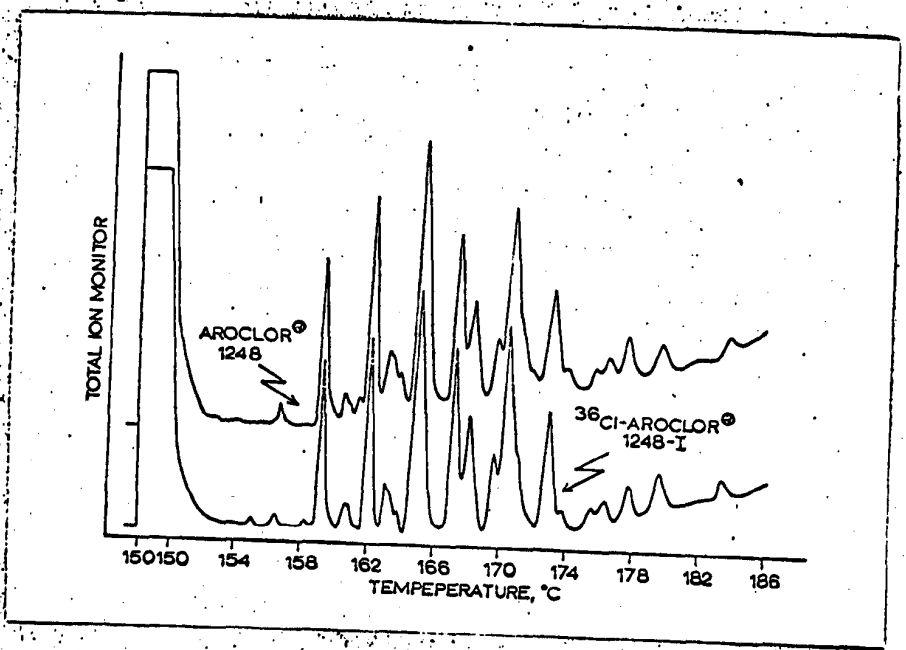


Fig. 4

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Figure 5. SCOT GC-MS comparison of Aroclor® 1254 and the radioactive Aroclor® 1254 material eluted in fraction I of the silicic acid cleanup. Chromatographic conditions are described in Figure 1.

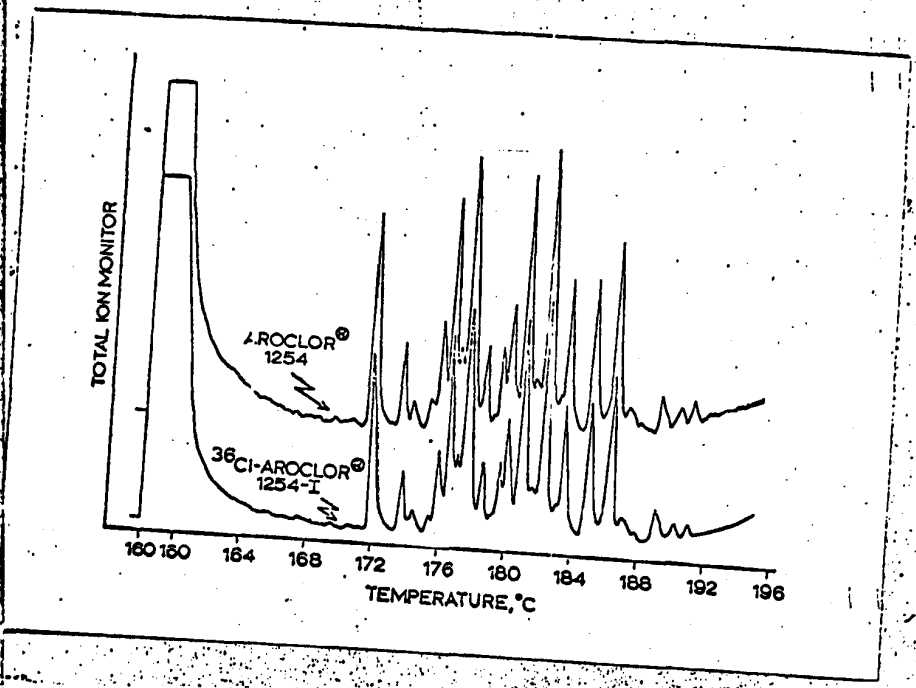


Fig. 5

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