Journal of Chromatography, 197 (1980) 155–169
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,920

GENERAL APPROACH TO THE FRACTIONATION AND CLASS DETERMINATION OF COMPLEX MIXTURES OF CHLORINATED AROMATIC COMPOUNDS

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(First received January 31st, 1980; revised manuscript received April 29th, 1980)

SUMMARY

Among the "inadvertent" environmental pollutants are polychlorinated biphenyls, terphenyls, quadphenyls, naphthalenes, diphenyl ethers, dibenzofurans, dibenzo-*p*-dioxins and benzenes. Mixtures of these classes of compounds also occur in commercial products such as transformer fluids. To analyze such mixtures, gas chromatography–mass spectrometry may be combined with pre-fractionation on basic and acidic alumina columns and semi-quantitative perchlorination techniques. These procedures are illustrated for synthetic mixtures as well as for two samples of stored transformer fluid. Although the described procedure is mainly intended to be applied to the characterization of the major class components of such mixtures, it is also applicable to the determination of trace components such as the dibenzofurans in commercial polychlorinated biphenyls. The mass spectral techniques permit the simultaneous patterning, or "fingerprinting", of the compounds comprising each major class of chlorinated aromatics present.

INTRODUCTION

The environmental chemist is often faced with the necessity of analyzing materials containing hundreds of individual components. For example, the commercial preparations of polychlorinated biphenyls (PCBs), used as heat exchangers, plasticizers, dielectric fluids, etc., usually consist of 40–100 individual compounds[1–3]. Many products are even more complex, in that mixtures of classes of compounds are present. An environmental sample may have been contaminated by PCBs, polychlorinated naphthalenes (PCNs), polychlorinated terphenyls (PCTs), pesticides, chlorinated benzenes (CBs), polychlorinated dibenzofurans (PCDBFs) and/or dibenzo-*p*-dioxins (PCDBDs), as well as other halogenated analogues and alteration products. Although the major interest of environmentalists in these products usually centers around their content of specific compounds having extreme toxicity, carcinogenicity or other undesirable biological activity, direct analysis for specific compounds is usually impracticable. These various classes of halogenated aromatics mutually inter-

fere with routine assay procedures[3–5], and some form of preliminary class fractionation is needed. To be practical, such a fractionation scheme must be applicable in the absence of previous knowledge of the class composition of the sample.

The purpose of the present work was to develop and characterize such a prefractionation scheme, to combine it with methods for the "fingerprinting" of the particular classes present, and to begin to apply it to actual environmental samples. To illustrate the effectiveness of the proposed procedure, we have applied it to the characterization of two samples of transformer fluid which differed in complexity. The complete procedure requires approximately 100 μg of aromatic material after extraction and cleanup. However, this may correspond to as little as 1 μg of any individual compound when complex mixtures such as PCBs are present.

EXPERIMENTAL

Materials

The following chromatographic adsorbents were used throughout this work: Florisil PR, 60–100 mesh (no lot number given) from Supelco (Bellefonte, PA, U.S.A.); basic alumina, 80–200 mesh, Fisher No. A-540 (lot No. 783941; Fisher Scientific, Pittsburgh, PA, U.S.A.); acidic alumina, 80–200 mesh, EM No. 1078 (lot No. 690691; EM Labs., Elmsford, NY, U.S.A.); and silica gel 60, 70–230 mesh, EM No. 7734 (lot No. 7956928; EM Labs.). Although we have not seen any significant lot number variations, the substitution of other types of alumina, Florisil or silica gel would require considerable modification of the procedures to be described. These adsorbents were all activated at 130°C; activation periods between 16 and 72 h gave comparable results, but shorter or longer heating periods, should be avoided.

Granular, anhydrous sodium sulfate was Fisher No. S-421. Sea sand was 50 mesh Sargent-Welch (Skokie, IL, U.S.A.) No. S-73845-8. Chromatographic columns were essentially similar to uncalibrated burettes, equipped with PTFE stopcocks, and plugged with Pyrex glass wool. Sintered glass frits caused adsorption problems and were avoided. Columns having an I.D. of 7 mm were used with 1–2 g of packing; 9 mm I.D. columns were used for 2–4 g of adsorbent; 1 cm I.D. columns for up to 7 g, and 1.5 cm I.D. columns for 7–10 g of adsorbent.

All solvents were distilled-in-glass, either from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) or Matheson, Coleman & Bell (East Rutherford, NJ, U.S.A.). All except methylene chloride and *n*-hexane were used as received; the former was stored over molecular sieve 5A and the latter over activated Davidson type 06 silica gel to ensure dryness. Concentrated sulfuric acid was reagent grade from Mallinckrodt (St. Louis, MO, U.S.A.). Perchlorination reagents sulfuryl chloride, sulfur monochloride and aluminum chloride were from Aldrich (Milwaukee, WI, U.S.A.). Antimony pentachloridewas from Alfa division of Ventron (Beverly, MA, U.S.A.); lot No. 112275 was free of significant bromide contamination[6,7].

Adsorption chromatography was monitored using either an Isco Model UA-5 UV monitor or an LDC No. 1280 monitor set at 280 nm. Fractions were collected on a drop-count basis. Infrared spectra of neat films on sodium chloride plates were run using a Perkin-Elmer Model 621 instrument.

Gas chromatography (GC) was performed using a Varian Model 1200 instrument equipped with a hydrogen flame ionization detector (FID), or a Hewlett-

Packard Model 5750 with a ^{63}Ni electron capture detector (ECD) in the pulse mode with 5% methane in argon as make-up gas. Peak areas were measured using an Autolab system IV computing integrator. Column packings for GC were from Applied Science Labs. (State College, PA, U.S.A.) or from Analabs (North Haven, DE, U.S.A.).

Compounds used as reference standards were obtained from a variety of sources. Chlorobenzenes were from Aldrich or Eastman-Kodak (Rochester, NY, U.S.A.); individual PCBs, polychlorinated diphenyl ethers (PCDPEs), hydroxy PCBs, PCDBDs and the like were from either Analabs or RFR (Hope, RI,, U.S.A.). Various PCBs, PCDPEs and PCDBFs were synthesized here by methods described previously[8,9]. Aroclors 1016, 1221, 1242, 1248, 1254 and 1260 were kindly donated several years ago by Monsanto (St. Louis, MO, U.S.A.). Aroclor 5460 (a mixture of polychlorinated terphenyls), various Halowaxes (mixtures of PCNs), and Kanoclor 400 (PCBs) were from Analabs. A mixture of polychlorinated diphenyl ethers having from 5–8 chlorine atoms per molecule was synthesized by reacting diphenyl ether (Eastman-Kodak) with eight equivalents of antimony pentachloride at 155°C in a sealed tube with chloroform as solvent, for 16 h. Polychlorinated quadphenyls (PCQs) were made by heating Kanoclor 400 (1 g) and activated copper powder (300 mg) under nitrogen in a sealed ampoule at 300°C for 48 h. The PCQs could easily be separated from PCBs on silica gel; PCBs were eluted with hexane, and PCQs with 5% diethyl ether in hexane. Fully perchlorinated compounds were obtained from Analabs or RFR. Samples of "used" transformer fluids were obtained locally.

GC–mass spectrometry (MS) was performed using a Finnigan 3300 mass spectrometer which was interfaced to a Finnigan/Incos 2300 data system. The ionization mode was electron impact at 70 eV and the separator temperature was 270°C. A 4-sec scan cycle was used throughout the GC–MS runs. Exact mass measurements were made using a VG Micromass ZAB-2F double focusing mass spectrometer at a resolution of 5000.

Methods

Preliminary enrichment. Commercial products may either enter the fractionation scheme (Fig. 1) directly if they are exclusively or predominately composed of halogenated aromatic compounds, or, if significant amounts of aliphatics are present they may be subjected to gel permeation chromatography (GPC)[10,11] or partitioned between isooctane and dimethyl sulfoxide[12] to provide a fraction highly enriched in aromatics. Environmental samples (soil, tissues, etc.) are first extracted by a procedure appropriate to the matrix (extraction methods are beyond the scope of the present paper). The extracts are largely freed of lipids and other aliphatics either by GPC[10,11,13] or by partitioning between carbon tetrachloride and sulfuric acid[14].

In order to remove the solvent in which the sample is at this point contained, we add 10–50 μl of 1,3-propanediol and concentrate at 40°C using a rotary evaporator. We observe no loss of PCBs from Aroclor 1254 if this precaution is taken; in the absence of the propanediol, up to 60% of the PCBs may be lost upon rotary evaporation.

The residue from rotary evaporation is taken up in 2 ml of *n*-heptane and washed with 5 ml of water to remove the propanediol. The upper phase, after centrifugation, is ready for fractionation according to the scheme shown in Fig. 1.

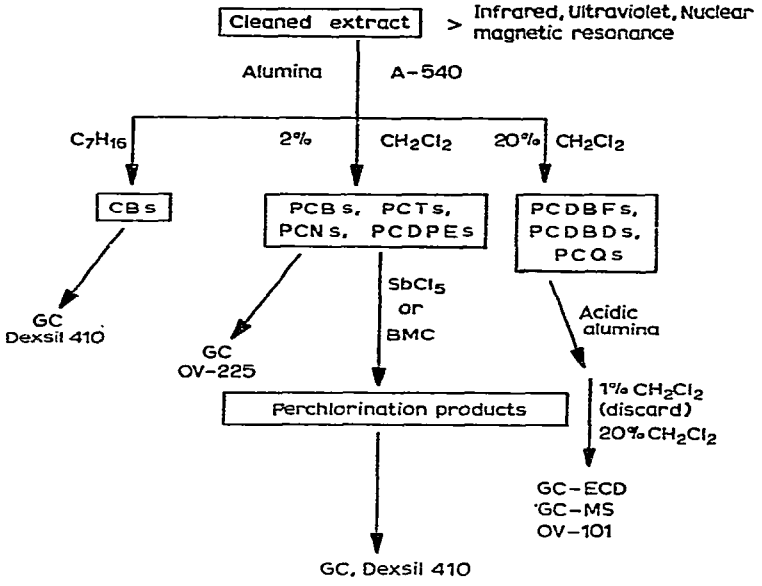

Fig. 1. Routine fractionation scheme.

Fractionation. The initial chromatography on A-540 basic alumina requires 5 g of alumina per mg of halogenated aromatic material present in the sample. The column is dry-packed with alumina (previously activated at 130°C) as soon as the alumina has cooled to room temperature in a desiccator over Drierite. The packing is settled by tapping gently (not tamping). A well-packed column will contain 1 g of alumina per 1.15 ml of packed volume. The void volume will be approximately 0.77 ml per gram of alumina. Before loading the sample, the column is topped with a 2 cm layer of anhydrous, granular sodium sulfate.

The sample is loaded in *n*-heptane, using less than 1 ml of solvent per 3 g of alumina in the column. As soon as the solvent has sunk to the surface of the sodium sulfate, the stopcock is closed for 30 sec to permit binding to become complete. Then the elution of the first fraction may be started, using the first two ml of *n*-heptane to rinse the sample container.

The amount of solvent needed to elute a given fraction depends upon the composition of the sample. In most cases, the first fraction (aliphatic hydrocarbons and chlorobenzenes) will be eluted by the time 3–4 ml of heptane per gram of alumina has passed through the column; however, PCBs and PCNs will begin to elute immediately after the chlorobenzenes and a sharp separation will require that the effluent be continuously monitored for UV absorbance. As soon as the emergence of a second UV-absorbing peak is detected, the eluting solvent is changed to *n*-hexane–methylene chloride (98:2, v/v). It is essential that the second solvent does not contain more than 2% methylene chloride if PCDBFs are to be separated from PCBs.

If the amount of aromatic material available is too small for UV monitoring, the alumina column can be precalibrated by running 50 μg of 2,3,5,6,2′,3′,5′,6′-octachlorobiphenyl in *n*-heptane. This compound will permit detection of the eluate volume corresponding to first appearance of PCBs, and can be completely eluted

with heptane. Since heptane (dry) does not deactivate the column, the calibrated column can then be used to process the low-level unknown sample. The elution solvent will be changed to 2% methylene chloride when an amount of heptane corresponding to the PCB breakthrough volume has been collected.

In general, the amount of 2% methylene chloride in hexane that will be required is at least twice that of the heptane used in eluting chlorobenzenes. If only the more highly chlorinated PCBs are present (analogous to Aroclor 1254 or 1260), fraction 2 will require 10 ml of 2% methylene chloride per gram of alumina. Less chlorinated PCBs (mono-, di- and trichloro) will require 20 ml/g, while PCTs would require 40 ml/g. Unfortunately PCDBFs will begin to elute if more than 10–15 ml of 2% methylene chloride per gram of alumina is used. If both PCTs and PCDBFs are present it will be necessary to run two columns, one from which PCTs are eluted in fraction 2 with 40 ml of 2% methylene chloride per gram of alumina (in which case PCDBFs will be impossible to purify) and one from which only a portion of the PCTs are eluted in fraction 2 with 10 ml solvent per g alumina so that the PCDBF fraction may be eluted and further purified.

Fraction 3, containing PCDBFs, PCDBDs, PCQs and part of the PCTs, is eluted with *n*-hexane–methylene chloride (80:20, v/v), 10 ml/g alumina. This fraction is further purified, to remove small amounts of the less chlorinated PCBs and PCNs, on a small (usually 1–3 g) column of acidic alumina prepared as described for the A-540 alumina. A well-packed column of 70–230 mesh acidic alumina will occupy a volume of 1.06 ml per gram of packing and have a void volume of 0.74 ml per gram. Fraction 3 from the basic column can generally be blown dry under nitrogen with no loss of its contents, since quadphenyls and the cyclic ethers are much less volatile than the PCBs. The residue is loaded onto the acidic alumina in *n*-hexane–methylene chloride (99:1, v/v) and eluted with 10 ml of the same solvent per gram of alumina. The purified PCQ–PCDBF–PCDBD fraction is then eluted with 6 ml of *n*-hexane–methylene chloride (80:20, v/v) per gram of alumina and blown dry under nitrogen at room temperature.

More polar materials may be recovered from the A-540 alumina column if desired. Phthalate diesters are eluted as fraction 4 with *n*-hexane–methylene chloride (1:1, v/v), 10 ml/g alumina. Phenols can be eluted with acetone, but recovery is poor. If the sample is expected to contain phenols, a portion should be run initially on acidic alumina, discarding (or combining) the fractions eluted through 20% methylene chloride, and eluting the phenols with acetone. Better recoveries of phenols will be obtained if the column is run in the dark (*e.g.* wrapped in aluminum foil) and all solvents are deoxygenated before use.

Analysis of the chlorobenzenes (fraction 1). Conditions used for GC of all the fractions are given in Table I. Dexsil 410 gave reasonably acceptable resolution of the various chlorobenzene isomers, although there were two unresolved pairs (Table II). Accurate quantitation with an ECD requires that a standard curve be prepared for each component. The hydrogen FID decreases in sensitivity as the degree of chlorination increases, but the response is nearly independent of the location of the chlorine atoms, so only six standard curves are needed.

Analysis of fraction 2. Fraction 2 commonly contains PCBs, PCNs, PCDPEs, DDE and part or all of the PCTs. Whether or not this mixture of classes can be further resolved into individual classes depends upon the complexity of each class. In general,

TABLE I

GAS CHROMATOGRAPHY CONDITIONS

Parameter	*Chlorobenzenes*	*PCB, PCN, PCDPE*	*PCDBFs, PCDBDs*	*PCT, PCQ*	*Perchlorination products*
Column length (m)	2	2	2	1	2
Column diameter (mm)	2	2	2	2	2
Liquid phase	Dexsil 410	OV-225	OV-101	OV-210	Dexsil 410
Percentage liquid	3	3	5	0.4	3
Solid support	Anakrom AS	Gas-Chrom Q	Gas-Chrom Q	TGB*	Anakrom AS
Mesh range	90–100	100–120	100–120	80–100	90–100
Helium flow-rate (ml/min)	35	25	20	25	35
Column temperature (°C)					
Initial	80	150	220	200	120
Final	150	250**	—	275	250
Linear rate (°C/min)	2	6	—	6	6
Initial hold (min)	3	—	—	—	—

* TGB = textured glass beads.

** To 300°C if PCTs are present.

TABLE II

GC OF CHLOROBENZENES ON DEXSIL 410

GC conditions as described in Table I. Samples of transformer fluid, heptane fraction from basic alumina. ND = none detected.

Component	*Retention*		*FID area (%)*	
	Time (min)	*Temp. (°C)*	*Fluid A*	*Fluid B*
Monochloro	0.86	80.0	ND	ND
m- + *p*-dichloro	2.44	80.0	0.26	0.73
o-dichloro	3.20	80.8	0.62	0.64
1,3,5-Cl_3	6.32	93.3	0.13	trace
1,2,4-Cl_3	7.10	96.4	75.23	67.23
1,2,3-Cl_3	8.60	102.4	23.75	12.30
1,2,3,5 + 1,2,4,5-Cl_4	11.82	115.3	trace	2.32
1,2,3,4-Cl_4	12.90	119.6	ND	13.51
1,2,3,4,5-Cl_5	17.80	139.2	ND	2.32
Hexachloro	23.62	162.5	ND	0.95

we find it more practical to functionally distinguish members of each class by MS techniques than to proliferate fractionation procedures with their attendant handling losses. The GC conditions used for introduction of the fraction 2 components are shown in Table I. Electron impact (70 eV) mass spectra are continuously generated in the scanning mode, after which the computer is used to generate reconstructed, single-ion chromatograms at each of the *m/z* values shown in Table III. These *m/z* values correspond to the molecular ion containing one ^{37}Cl atom and show much less cross-class interference than the all-^{35}Cl molecular ion peaks. Selectivity was further increased by only considering the peaks seen in specific scan number ranges for each single ion chromatogram, as also shown in Table III. In this way interference from the ($M - Cl_2$) fragment from higher analogues could be avoided. The appropriate ranges were determined from chromatograms of various Aroclor, Halowax, and PCDPE mixtures.

TABLE III

m/z VALUES AND SCAN RANGES FOR RECONSTRUCTED SINGLE ION CHROMATOGRAMS

Scan numbers are at 4 sec per scan.

PCN	*m/z*	*Scan numbers*	*PCB*	*m/z*	*Scan numbers*	*PCDPE*	*m/z*	*Scan numbers*
3-Cl	232	1–60	4-Cl	292	40–82	6-Cl	376	80–120
4-Cl	266	30–90	5-Cl	326	60–110	7-Cl	410	110–140
5-Cl	300	60–110	6-Cl	360	80–140	8-Cl	444	140–180
6-Cl	334	90–150	7-Cl	394	95–200	9-Cl	478	180–200

Since the composition of fraction 2 is commonly much too complex to permit quantitation on the basis of standard curves for individual compounds, only approximate methods of quantitation could be used. The relative peak areas for the PCDPEs correlated well with corresponding relative hydrogen FID peak areas, which

by analogy with previous observations on PCBs[15] were tentatively considered to approximate molar percentages. The single ion peak areas were related to the flame detector areas by: single ion peak area = 0.58 (hydrogen FID area) + 0.45, with a correlation coefficient of 0.9888.

Relative peak areas from the reconstructed single ion chromatograms for a variety of PCB standards unfortunately did not correlate well with the corresponding FID peak areas. However, the single ion relative molar responses for PCBs correlated well with the total number of chlorines, such that the relative molar responses (RMR) fit the equation: RMR = 0.132 (No. of Cl) + 0.202 with a correlation coefficient of 0.9956 ($n = 8$). This equation applied when RMR for 2,4,5,2′,5′-pentachlorobiphenyl was set equal to 1.0.

We did not have a collection of individual PCNs or PCTs available, so quantitation of individual components of these classes was not attempted. Considered over a variety of mixtures of Aroclors, Halowaxes and PCDPEs, on the average the total peak area attributed to PCDPEs × 3.4, that due to PCNs × 0.8 and that attributed to PCBs × 1.14 (from the single ion chromatograms) gave relative molar percentages of each class. These correction factors allowed only a first approximation of the class composition, of course, and, except for PCNs, more satisfactory measurements could be made by perchlorination of fraction 2. In general, the reconstructed single ion chromatograms were most useful as "fingerprint" patterns of class composition. PCTs do not begin to elute from Dexsil 410 until after scan 200 (about equivalent to the beginning of elution of nonachlorobiphenyls), and are thus in a region of the chromatograms reasonably free of interferences. Any *m/z* value in the molecular ion cluster can be used to monitor PCTs.

Perchlorination of fraction 2. Fraction 2 was routinely divided into three portions depending on the amount of material available. One portion was reserved for GC–MS as discussed above. Another portion, which could contain between 50 μg and 1 mg of total organic material after removal of solvent, was dissolved in 0.1 ml of chloroform and perchlorinated with 0.2 ml of antimony pentachloride for 16 h at 170°C as described by Armour[16]. This procedure quantitatively generates decachlorobiphenyl, the three isomeric tetradecachloroterphenyls and hexachlorobenzene from PCBs, PCTs and chlorobenzenes respectively, but destroys PCNs and PCDPEs. Mirex is not affected by perchlorination and may be added as an internal standard.

Perchlorination of the final portion of fraction 2 was performed using "BMC Reagent" (sulfuryl chloride, sulfur monochloride and aluminum chloride)[17]. To the dried residue containing less than 1 mg of aromatic compounds in a 15 ml screw cap test tube was added 0.5 ml of sulfuryl chloride, 10 μl of sulfur monochloride and 10–20 mg of aluminum chloride. It is essential that the aluminum chloride not be exposed to atmospheric moisture for more than a few seconds, and the final concentration of aluminium trichloride should not exceed 5%. The tubes were closed with PTFE-lined screw caps, and placed in a 70°C bath such that only the portions of the tubes containing reagent were immersed. After heating for 3 h, the tubes were cooled, opened, and the samples worked up as described in the original reference[17]. Again, Mirex was used as internal standard.

GC analysis of the perchlorination products under the conditions described in Table I is illustrated using standards in Fig. 2. Relative detector responses to the various pure components are indicated in Table IV.

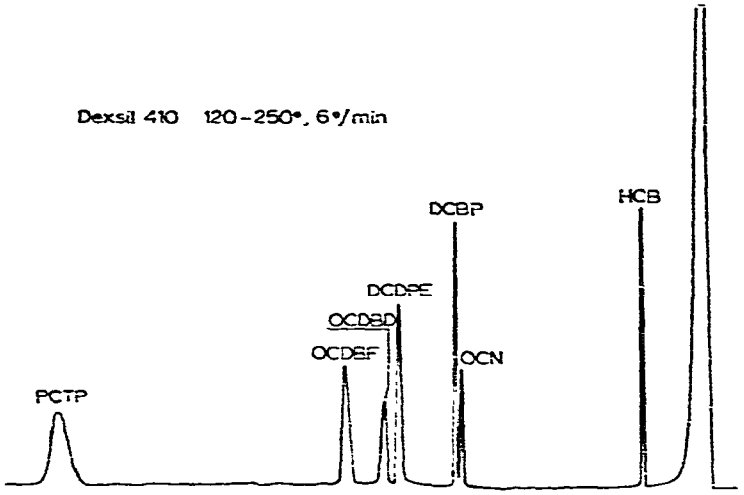

Fig. 2. Gas chromatogram of fully perchlorinated compounds on Dexsil 410. HCB = hexachlorobenzene; OCN = octachloronaphthalene; DCBP = decachlorobiphenyl; DCDPE = decachlorodiphenyl ether; OCDBD = octachlorodibenzo-*p*-dioxin; OCDBF = octachlorodibenzofuran; PCTP = tetradecachloroterphenyl (*para* isomer). OCN may elute before or after DCBP depending upon the column length under these programming conditions.

If the amount of available sample is below 50 μg, it will probably be undesirable to perform both perchlorination procedures. In such a case, the entire residue of fraction 2 not used for MS, augmented with 1 μg of Mirex, is processed according to the antimony pentachloride procedure, using 50 μl of chloroform and 0.1 ml of antimony pentachloride in a 1 ml Reactivial. Since chlorobenzenes have been separated in fraction 1, the occurrence of hexachlorobenzene in the perchlorination products will suggest that diphenyl ethers and/or naphthalenes were present in fraction 2 prior to their decomposition by the reagent. Further information on these classes of compounds would then have to be derived from the MS data.

Analysis of the 20% methylene chloride fraction. Quadphenyls do not elute from GC columns in the range of the PCDBFs or PCDBDs and may be examined

TABLE IV

HYDROGEN FLAME IONIZATION DETECTOR RESPONSE TO PERCHLORINATION PRODUCTS

Response is based on relative peak areas.

Compound	*Relative molar response*	*Relative weight response*
Mirex	1.000	1.000
Hexachlorobenzene	0.775	1.498
Octachloronaphthalene	1.032	1.395
Decachlorobiphenyl	0.979	1.080
Octachlorodibenzo-*p*-dioxin	0.473	0.561
Octachlorodibenzofuran	0.620	0.769
Decachlorodiphenyl ether	1.192	1.274
Tetradecachloroterphenyl	1.446	1.118

without interference by GLC on the low-loaded column described in Table I. If PCTs are absent, the PCDBFs and PCDBDs can be examined using the OV-101 column and an ECD, in which case quantitation will be based upon individual standard curves. The complete families of PCDBF and PCDBD isomers have not been successfully resolved, even using capillary columns[18,19]. Selectivity may be achieved through the use of negative ion chemical ionization MS[20], but because of the inability to distinguish all possible isomers it is necessary to limit characterization to chemical class and number of chlorine atoms in most cases. The occurrence of interferences on the OV-101 column will usually reflect failure to completely eliminate PCBs and/or PCTs, and may be grossly detected by taking note of the peak widths. The PCBs and PCTs give much narrower peaks than do the PCDBFs and PCDBDs at a given elution position.

APPLICATIONS AND ILLUSTRATIONS

Synthetic mixtures

A mixture of Aroclor 1254 (PCBs), Halowax 1013 (PCNs), and PCDPEs (synthetic mixture with 5–8 chlorine atoms per molecule) gave only a single, asymmetrical peak on A-540 alumina (fraction 2, 2% methylene chloride). This mixture was subjected to GC–MS and perchlorination as described above. Reconstructed single ion chromatograms are shown in Fig. 3, while the yields of fully perchlorinated products are given in Table V.

The yields of decachlorobiphenyl were essentially quantitative when the antimony pentachloride procedure was used. PCNs and PCDPEs were broken down to small fragments under these conditions. Yields were incomplete for decachlorobiphenyl and decachlorodiphenyl ether using the BMC reagent, and this reagent has also been found to be unsuitable for derivatizing PCTs. However, in all experiments to date, PCDPEs have been derivatized by the BMC reagent to the same extent as the PCBs; therefore the application of antimony pentachloride and the BMC reagent to separate aliquots of a sample permits the determination of PCDPE content.

Chlorinated naphthalenes break down to a variety of products including hexachlorobenzene when the perchlorination conditions are too vigorous. This occurs, in BMC reagent, if the aluminium trichloride concentration exceeds 5% (w/v). Under the conditions described above, with 2–5% aluminium trichloride, the yields of octachloronaphthalene approach 90%, depending upon the degree of protection of the reagent from atmospheric moisture. This is difficult to reproduce, and we do not presently recommend the use of perchlorination as a strictly quantitative assay for PCNs.

A mixture of Aroclor 1254, Aroclor 5460 (PCTs) and 1,2,3,4-tetrachlorobenzene gave the chromatographic pattern on A-540 alumina shown in Fig. 4. Perchlorination with antimony pentachloride gave essentially quantitative conversion of all three classes of compounds to their fully chlorinated analogs.

The application of these chromatographic procedures in association with chemical ionization negative ion MS to determine the chlorinated dibenzofuran content of commercial PCBs has been described previously[3] and will not be repeated here. In general, the non-oxygenated classes, PCBs, PCTs, etc. do not interfere with determination of the dibenzofurans in this mass spectral technique, so the contamination of fraction 3 by PCTs is not a serious problem. The use of both basic and acidic

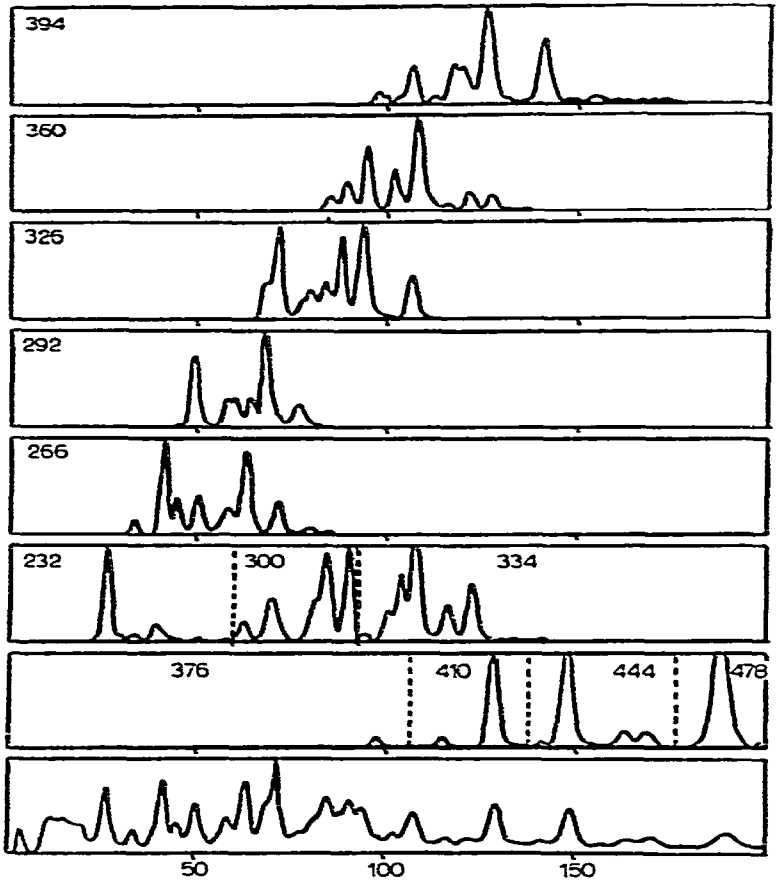

Fig. 3. Reconstructed single ion chromatograms for a mixture of PCBs, PCNs, and PCDPEs. The lowest trace is the total ion current. For GC conditions see Table I.

alumina columns in sequence is, however, essential if PCDPEs are present in the sample, since these compounds yield M—Cl_2 fragments that interfere with the determination of the dibenzofurans.

TABLE V

YIELDS OF PERCHLORINATION PRODUCTS FROM A MIXTURE

Theoretical yield is given as mean ± S.D. ($n = 5$).

Component	*Class*	*nmoles per sample*	*Theoretical yield of perchloro derivative (%)*	
			$SbCl_5$	*BMC*
Aroclor 5460	PCT	460	92.4 ± 3.6*	0
Aroclor 1254	PCB	654	95.9 ± 2.1	73.5 ± 6.5
Halowax 1013	PCN	843	0	c**
PCDPE (5–8 Cl)	PCDPE	516	0	74.9 ± 5.1

* Sum of *o*-, *m*- and *p*-isomers.

** Critically dependent upon the amount and activity of the aluminum chloride. Yields of from 0–98% have been seen.

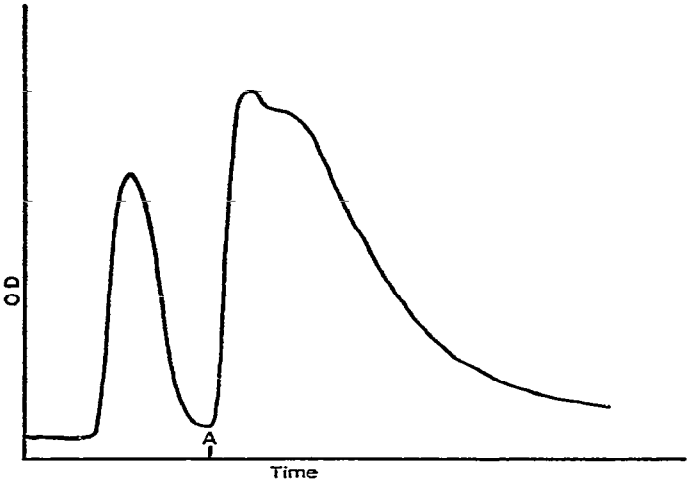

Fig. 4. UV monitor output during chromatography of a mixture of CBs, PCBs, and PCTs on A-540 alumina. Approximately equal amounts of 1,2,3,4-tetrachlorobenzene, Aroclor 1254 and Aroclor 5460 were chromatographed as described in the text. Solvent changed from heptane to 2% methylene chloride in hexane at point A.

Transformer fluids

Infrared examination of the two samples of transformer fluid indicated the absence of significant aliphatic components; accordingly, the samples could enter the analytical scheme at the basic alumina chromatography step. No C=O or –OH was detectable in the infrared spectra, indicating that phenolic compounds and phthalate esters would not comprise as much as 1% of the material. Therefore, only the initial three fractions from the alumina columns were studied in detail.

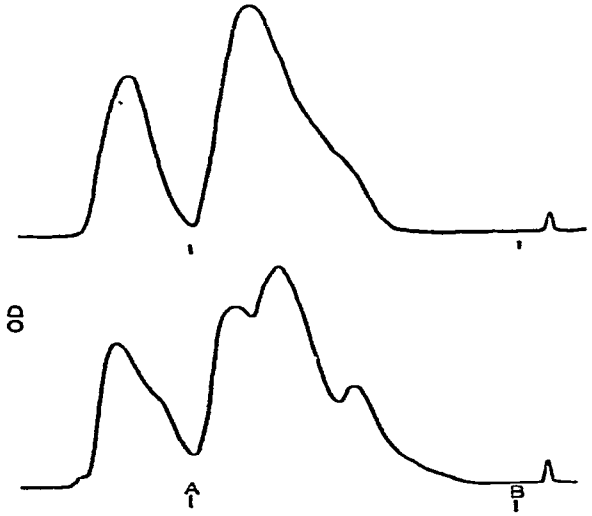

Fig. 5. UV monitor output during chromatography of transformer fluids on A-540 alumina. Upper trace, fluid A; lower trace, fluid B. Solvent changed from heptane to 2% methylene chloride in hexane at A, and to 20% methylene chloride in hexane at B.

The UV monitor outputs for the chromatography on A-540 alumina are shown in Fig. 5. Major elution peaks appeared in the heptane and the 2% methylene chloride fractions, with minor peaks in the 20% methylene chloride fraction.

Fluid A, fraction 1 (heptane), contained exclusively chlorobenzenes as shown in Table II. Fraction 2 contained PCBs similar in chromatographic pattern to Aroclor 1254. Other classes of compounds amounting to more than 0.1% of the sample weight were not detected. Fraction 3 (20% methylene chloride) from the A-540 alumina, after further cleanup on acidic alumina, contained a range of PCDBFs in trace amounts consistent with the reported PCDBF content of Aroclor 1254[21]. These PCDBFs were mainly tetra- and pentachloro isomers (Table VI), as would be expected.

TABLE VI

CHLORINATED DIBENZOFURANS IN TRANSFORMER FLUIDS

Upper limits measured with an ECD. ND = not detected.

No. of Cl	*Fluid (ng/g)*	
	Fluid A	*Fluid B*
4	580	ND
5	115	ND
6	45	16
7	ND	200
8	ND	4500

Fluid B was more complex than fluid A. Fraction 1 contained a wider range of chlorobenzenes than was seen in fluid A (Table II). Perchlorination of fraction 2 from fluid B gave decachlorodiphenyl ether in addition to decachlorobiphenyl. The chlorinated dibenzofuran in fraction 3 were not only in higher total concentration in fluid B than in fluid A, but were also more highly chlorinated than those in fluid A. The compositions of the two samples of transformer fluid are summarized in Table VII.

TABLE VII

COMPOSITIONS OF TWO SAMPLES OF TRANSFORMER FLUID

ND = none detected.

Component	*Fluid A*		*Fluid B*	
	Mol.%	*Pattern*	*Mol.%*	*Pattern*
PCBs	60.3	1254*	50.8	1260*
Chlorobenzenes	39.7	2–3 Cl	41.1	1–6 Cl
PCDPEs	ND	ND	8.0	6–8 Cl
PCNs, PCTs	ND	ND	Trace	—
PCDBFs, PCDBDs	ND	ND	ND	ND

* PCB pattern given as the Aroclor it most nearly resembled.

To confirm the identification of diphenyl ethers in fluid B, exact mass measurements were made on three peaks in the molecular ion cluster of the best resolved components of this class. The results are shown in Table VIII. In addition, this fraction was treated with the silylation reagent described above under conditions

giving quantitative silylation of 2,3,4,5,6,3',5'-heptachloro-4'-hydroxybiphenyl to check for the occurrence of hydroxybiphenyls. No silyl-reactive components were detected. MS properties of trimethylsilyl derivatives of hydroxybiphenyls have been described[22].

TABLE VIII

EXACT MASS MEASUREMENTS ON COMPONENTS OF TRANSFORMER FLUID B

Transformer fluid B had been in service for at least 20 years prior to sampling.

Scan No.	*Formula*	*No. of ^{37}Cl*	*Exact mass*		*Error (ppm)*
			Theory	*Measured*	
97	$C_{12}H_4OCl_6$	0	373.8393	373.8412	5
		1	375.8364	375.8362	1
		2	377.8334	377.8334	0
127	$C_{12}H_3OCl_7$	0	407.8003	407.8025	5
		1	409.7974	409.7981	2
		2	411.7944	411.7938	2
149	$C_{12}H_2OCl_8$	0	441.7614	441.7600	3
		1	443.7584	443.7612	6
		2	445.7555	445.7561	1

DISCUSSION

The classes of aromatic compounds discussed here, PCBs, PCNs, PCDPEs, PCTs, PCQs, PCDBFs, PCDBDs and CBs, represent "inadvertent" pollutants in that, unlike the pesticides and herbicides, they have not been deliberately disseminated in the environment. The PCDBFs and PCDBDs have entered the environment as unintentional impurities in PCBs and products derived from chlorophenols, as well as being pyrolysis products of PCBs and possibly PCDPEs under appropriate conditions[23,24]. The PCTs are rarely found as environmental pollutants, possibly because they are rarely sought. PCQs and higher "polymers" of PCBs are found in used heat exchange fluid such as lead to the "Yusho Oil" contamination in Japan[25]. Many commercial PCB preparations reportedly contain traces of PCNs[26], and the Halowaxes, mixtures of PCNs, share many of the applications of the PCBs and thus are likely to be found in similar matrices. At the present time, means to adequately determine concentrations of total PCNs in the presence of excess PCBs do not exist (for some of the problems involved see ref. 3).

Throughout the work presented here we have emphasized use of the hydrogen FID rather than the ECD. Not only is the response of the FID much more uniform and predictable than that of the ECD, but the flame sensitivity is closely comparable to that of the mass spectrometer in the scanning mode. Any sample not sufficiently cleaned up to use a FID is also not sufficiently clean for perchlorination. The minimum amount of *e.g.* a perchlorination product that can be accurately quantitated using the FID under the GC conditions described is approximately 5–10 ng at an amplifier setting of $16 \cdot 10^{-12}$ A/mV. This is quite adequate sensitivity relative to the limitations of the mass spectrometer and the obtainable purity of perchlorination reagents.

The procedures described here could give indications of the presence of aromatic compounds other than those specifically discussed. For example, polybromi-

nated biphenyls would show as a discrepancy between the apparent amount of PCBs seen by MS and by GC of the perchlorination products. However, we have not at this time specifically included methods for the separate quantitation of brominated or unsubstituted compounds. The effects of major quantities of pesticides in the samples have also not been examined. The nearly ubiquitous pesticide DDE, which also elutes in fraction 2 under the conditions described, can be determined without interference from PCBs, PCNs, PCTs or PCDPEs by monitoring its m/z 318 ion in the MS procedure. DDE yields no significant interference with any of the ions listed in Table III. PCTs would interfere with mass spectral determination of PCBs, were it not for the fact that they elute from the GC column after the PCB scans have been completed.

The use of chlorination procedures to screen for chlorodibenzo-*p*-dioxins in the presence of PCBs, PCNs and PCDBFs has been suggested previously[27]. In our experience, although the general principal seems reasonable, perchlorination reactions are not sufficiently "clean" to permit the quantitative determination of the trace components of these mixtures. Moreover, toxicological interest at the present time centers on a few specific PCDBF and PCDBD isomers; therefore we prefer to analyze for these classes of compounds following a cleanup procedure rather than a purely qualitative screening procedure that seems to offer considerable potential for false positives.

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