P C B N E W S L E T T E R

30 March 1972

MONS 077365

ANALYTICAL METHODS

AND

CHEMICAL CHARACTERISTICS

MONS 077366

David L. Stalling and James N. Huckins, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Silicic Acid PCB-Pesticide Separation Method

Using the Armour and Burke Silicic Acid-Celite® Column procedure for separation of PCBs from DDT and its analogs, some components of Aroclors® 1242, 1232, and 1221 elute in the pesticide fraction. To date, there has been little information available on the component distribution of the mentioned Aroclors in the PCB-pesticide fractions. In our analyses, Celite has often been found to be contaminated with a compound of unknown origin, which has a GC retention time near p,p'-DDT. Celite does not appear to enhance PCB-pesticide separation nor does it seem necessary in order to maintain an adequate column flow rate if a pressure head is used.

To overcome these problems inherent in the Armour and Burke Silicic Acid-Celite System, we have modified the method. Celite was eliminated and only 2% water (98g silicic acid + 2 ml water) is added to deactivate the silicic acid. In addition, because small amounts of benzene contaminants in the solvents affect elution, the PCB phase eluting solvent was changed by adding 0.1% benzene (v/v) to petroleum ether. The pesticide phase solvent was replaced with 20% (v-v) diethyl ether in benzene and we selected the optimum elution volumes for both phases. These modifications have permitted PCB-Pesticide® separations having the maximum number of components of Aroclors® 1242, 1232, and 1221 in the PCB fraction without eluting DDT and analogs and other similar pesticides in the PCB fraction.

Experimental

Reagents

(a) Silicic acid - Mallinckrodt, 100 mesh powder; Analytical Reagent No. 2848. Prepare as described by the Armour and Burke method with the following exceptions: activate for a minimum of 12 hrs at 130° and add 2% water (98g silicic acid + 2 ml water) with a pipette.

(b) Solvents used - petroleum ether, benzene, and diethyl ether suitable for pesticide analysis.

(c) Eluting mixture - PCB fraction (0-250 ml): 0.1% benzene, 99.9% petroleum ether (v/v). Pesticide fraction (250-450 ml): 20% diethyl ether, 80% benzene (v/v).

Apparatus

(a) Chromtographic column - 22 mm I.D. x 300 mm glass column with removable teflon stopcock, joined at the top with a 250 ml glass reservoir having a 24/40 standard female ground glass joint. A 24/40 male joint joined to a 1/2" short piece of heavy walled tubing is attached to an air outlet. This adapter is held in place on the column with a standard taper clamp (Thomas), and will maintain a pressure of about 5 lbs.

MCNS 077367

Procedure

Weigh 20 g of 2% silicic acid into a 250 ml beaker. Immediately slurry with 70 ml of pet ether and pour into chromatographic column with stopcock open. Rinse the silicic acid remaining in the beaker into the column with additional pet ether and rinse down sides of column. Allow the silicic acid to settle but do not allow the column to drain dry at any time. Next add 2 g of Na_2SO_4 to the top of the silicic acid.

Apply air pressure (2-3 psi) until the pet ether level in the column is about even with the top of the Na_2SO_4 layer. Add sample cleaned up with Florisil or gel permeation in not more than 5 ml of pet ether or iso-octane and apply pressure until sample level is even with Na_2SO_4. Wash down sides of the column with 5 ml of pet ether and bring solvent level even with Na_2SO_4 again. Place a 150 ml graduated beaker under the column and collect next rinse of 5 ml 0.1% benzene in pet ether. Then add about 275 ml of 0.1% benzene in pet ether to the column reservoir and apply air pressure until a flow rate of approximately 4 ml/min is achieved.

Collect the 0-100 ml eluant fraction and quickly place a graduated 400 ml beaker under the column while still eluting. Collect the next 100-250 ml fraction and stop elution. The solvent level should be close to the top of the Na_2SO_4 layer. Place another 400 ml graduated beaker under the column and add 200 ml of 20% diethyl ether in benzene to the column reservoir. Continue elution until the 200 ml mark is reached. This completes the separation.

Results and Discussion

The elution pattern using the method described above is as follows. The 0-100 ml PCB fraction will contain the first three components of technical chlordane, hexachlorobenzene (C_6Cl_6), mirex and some Aroclor® 1260. The 100-250 ml PCB fraction contains components of Aroclors® 1260, 1254, 1248, and most 1242 components, and some late GC eluting components of 1232 and 1221. Pesticides found in the PCB fraction (100-250 ml) are aldrin, heptachlor (also a component of technical chlordane), and some p,p'-DDE. The pesticide fraction (250-450 ml) will contain most of the components of Aroclor® 1221 and the first few early GC eluting components of Aroclors® 1232 and 1242. Using a mixture of Aroclors® 1260, 1242, and 1221 as a standard, the actual PCB component split centers around the fifth, sixth, or seventh PCB peak from the solvent peak using an OV-7 GC column. If PCB components with shorter GC retention time than peak #5 from the solvent peak elute in the PCB fraction (0-250 ml), most of the p,p'-DDE, o,p'-DDE, p,p'-DDT and o,p'-DDT will elute in the PCB fraction. (If the quantative separation of mirex from Aroclor® 1260 is desired, another 2% silicic acid column, using pet ether only as an eluting solvent, is necessary. Mirex elutes in the 0-100 ml fraction.)

Because silicic acid may vary in adsorptive capacity from one batch to another, it is advisable to run a mixture of Aroclor standards (1260, 1242, and 1221) along with the samples to be processed. This enables good definition of the Aroclor component eplit between PCB and pesticide fractions for a specific lot of silicic acid.

It was determined experimentally that additional small amounts of benzene which might be present as contamination in pet ether have little effect on the PCB elution pattern. We also found that 20% diethyl ether in benzene (v/v) as the pesticide eluting solvent gave recoveries similar to the pesticide eluting solvent described by Armour and Burke.

Reverse Phase Thin Layer Chromatography (RPTLC) of Aroclors® (PCBs), Halowaxes® and Pesticides

Reverse phase TLC has been used to resolve components of Phenochlor Dp6 or Aroclor® 1260 (R.H. DeVoe and E. W. Peat, Bull. Environ. Contam. Toxicol., 6, 164-170, 1971). RPTLC permits component separation by partition between a non-polar stationary phase and a polar mobile phase. Kieselguhr G plates (layer thickness 0.25 mm) were impregnated with liquid paraffin as the stationary phase. The components of Aroclor's® 1232, 1248, 1254, 1260; Halowaxes® 1099, 1013, 1014, and several chlorinated pesticides were resolved with one of four solvent systems (mobile phases); I-water, acetonitrile, and methanol (20:40:40); II-water, acetonitrile and methanol (15:40:45); III-water, acetonitrile and methanol (10:45:45); IV-water, acatone, acetonitrile and methanol (5:15:40:40). The R_f values for these materials were determined in solvent systems II and IV. Components of (RPTLC) spots were characterized by GC and/or GC-MS. In general, GC peaks with higher retention times corresponded to spots with lower (RPTLC) R_f values. Certain GC peaks were resolved into more than one component by RPTLC. RPTLC did not resolve aldrin and DDE from some components of PCB's and Halowaxes with similar R_f values. However, when the PCB components are recovered from the plate, they are easily separated from aldrin and DDE by GC. Research is presently underway on the application of RPTLC principles to column chromatography.

Experimental data presented here was collected by: Judith Armour, Bernadette McMahon, John Roach, Winfred Wiencke, and Susan Young of the Division of Chemical Technology; Herbert Masumoto and Milton Luke of the Los Angeles District; and compiled by Bernadette McMahon, Food and Drug Administration

New information on the composition of Aroclor references and revised data on the behavior of Aroclors in analytical procedures has been obtained in a series of short term studies in laboratories of the FDA. A GLC system capable of determining the polychlorinated terphenyl (PCT) components of certain Aroclors was developed and subsequently used to obtain the new and revised information on PCT.

Aroclor 1232 Reference Material

New information on the manufacturing process for Aroclor 1232 sheds light on the reason for the differences among the chromatograms of several samples of 1232 obtained from Monsanto at different times. It has been revealed that when Aroclor 1232 was being manufactured for sale (it is no longer available), it was made by combining amounts of Aroclors 1221 and 1242 until the mixture contained 32% chlorine by weight. This information explains how it is possible that the gas chromatograms of some lots of Aroclor 1232 reference materials have resembled chromatograms of 1242, and others have more closely resembled chromatograms of 1221.

It is probable that the manufactured lots of Aroclor 1232 varied in composition from one to another. For this reason, residues of Aroclor 1232 that may be found would be unlikely to match any arbitarily chosen 1232 reference material. It is therefore recommended that residues suspected of being "Aroclor 1232," but resembling Aroclor 1221 and/or 1242, be quantitated by comparison to Aroclor 1221 and/or 1242 reference materials, rather than by comparison to an Aroclor 1232 reference.

From now on, Aroclor 1232 will not be considered a separate entity by our laboratories, and lists of data on the behavior of Aroclors in this correspondence will not include Aroclor 1232.

Significance: Little Aroclor 1232 was ever sold (according to Monsanto) and none is available now, so residues of "1232" are not likely. The suggested means of quantitation is available only in case such a residue is found. Toxicological and ecological reasons for identifying the residue will not be jeopardized by reporting the residue as 1221 and/or 1242 rather than as 1232.

An EC-GLC System for the Determination of Polychlorinated Terphenyls and/or Polychlorinated Biphenyls

Winfred Wiencka

A two-column EC-GLC system has been devised to permit determination of both PCB and polychlorinated terphenyls (PCT) using only one dual-column oven. The use of the GLC columns commonly used for pesticide and PCB residue determination is impractical for PCT determination because of the extended elution times (up to several hours) and consequent poor sensitivity to the PCT with such columns.

A low load (1%) OV-101 column at 240°C permits elution of the PCT in Aroclor 5460 in less than 15 min and improves sensitivity so that one half scale response for 20 ng is readily attainable. A second column of 3% Dexsil, maintained in the same 240°C column oven, permits elution of the PCB in Aroclor 1260 in less than 10 min. Response to 1260 is greater than response to the same amount of 5460 when the two columns are tee'd into the same detector. It is also possible to utilize two EC detectors, one for each column.

Specific parameters of the system are:

OV-101 Column for PCT

Column: 6' x 1/4" glass, on column injection
Liquid phase: 1% w/w OV-101
Support: 100/120 mesh Gas Chrom Q or equivalent
Column oven temp: 240°C
N_2 flow rate: 120 ml/min
Injector temp: 250°C
Conditioning: 24 hours @ 270°C with a final hour at 300°C; N_2 purge at 10-30 ml/min
EC detector temp: 200°C

Dexsil 300 Column for PCB

Column: 6' x 1/4 " glass, on column injection
Liquid phase: 3% w/w Dexsil 300
Support: 100/120 mesh Gas Chrom Q or equivalent
Column oven temp: 240°C
N_2 flow rate: 60 ml/min
Injector temp: 250°C
Conditioning: 48 hrs. @ 270°C with a final 2 hrs. @ 300°C; N_2 purge at 10-30 ml/min
EC detector temp: 200°C

Electronics

Electrometer: 1 x 10^{-9} Amperes for full scale deflection (AFS)
Chart speed: 1/2" per min
EC Detector: Concentric type (Packard), H^3 foil

Aroclors 4465, 5442, and 5460 Reference Materials

By definition of the manufacturer, the Aroclors named in the title are composed of mixtures of the following:

4465 - chlorinated biphenyls and terphenyls, with 65% chlorine by weight

5442 - chlorinated terphenyls, with 42% chlorine by weight

5460 - chlorinated terphenyls, with 60% chlorine by weight

It is now apparent that when Aroclors 4465, 5442, and 5460 were chromatographed at conditions normally used for the chromatography of the mixtures of chlorinated biphenyls, the chlorinated terphenyls present in these mixtures were seen, at best, only as very late eluting peaks. In turn, this failure of the chromatographic system to visualize the terphenyls led to the reporting of some inaccurate data on these mixtures.

Recent investigations from several sources have revealed new information about the Aroclors in question. In part, the evidence has been obtained by use of the 1% OV-101 GLC column, which permits terphenyls to elute quickly enough to be easily seen and measured (see above).

New findings on the Aroclors of interest follow:

4465. The reference material available in our laboratories appears to be mostly biphenyl compounds, despite Monsanto's description of this mixture as 60:40 terphenyl: biphenyl. Chromatography of 4465 on a GLC column which permits early elution of terphenyls does not reveal the presence of additional significant compounds.

Because the 4465 reference material obtained from Monsanto by FDA does not match Monsanto's description of the product, it is suspected that the terphenyl: biphenyl proportion must vary among different manufactured lots. The description of the material as a combination of terphenyl and biphenyl also suggests that 4465 may have been made by combining amounts of other Aroclors until 65% chlorination was obtained. Therefore, it may be possible to handle residues of "4465" by comparing them to other Aroclor references which they resemble.

Monsanto no longer manufactures Aroclor 4465, so no additional samples of reference material are available. For this reason, data presented in other sections of this communication were obtained using the reference material containing mostly chlorobiphenyls.

5442. The reference material of 5442 available in our laboratories contains some contamination with chlorinated biphenyls, as evidenced by the early eluting peaks in the gas chromatograms obtained using GLC parameters "normal" for PCB determination. The contamination is relatively small, and the data for 5442 presented elsewhere in this communication was obtained by measuring the recovery of the terphenyls using the 1% OV-101 column (see above).

5460. The 5460 available in our laboratories has now been shown to contain a relatively large (about 5%) contamination of chlorinated biphenyls. It is now apparent that only the biphenyl contaminant was readily

visualised by the "normal" GLC systems, which explains why chromatograms of 5460 resembled 1260 so closely. The chlorinated terphenyl components of 5460 can be seen and measured when the 1% OV-101 GLC column is used (see above). Data on the behavior of 5460 presented elsewhere in this correspondence were obtained using the OV-101 column to visualize the terphenyl components of 5460 for measurement.

Significance: Residues of chlorinatad terphenyls are presumably of as much interest and importance as those of chlorinated biphenyls. Until recently, the capability for detecting and measuring chloroterphenyl residues was limited by the very long retention times and consequent poor sensitivity for the compounds on the GLC columns (PAM 311.2) normally used for analysis of chlorinated biphenyls.

The chlorinated terphenyls are recovered, at least partially, through the analytical procedures used for organochlorine pesticides and PCB (see other sections of this correspondence for details). It is now possible, by means of the 1% OV-101 GLC column described by Wiencke (see above), to visualize residues of chlorinated terphenyls and to estimate their quantity against a known amount of reference material. This is a recommended procedure for detection and measurement of residues in samples suspected of contamination with chlorinated terphenyls. In particular, samples whose preliminary GLC analysis shows peaks eluting beyond the retention times of chlorinated biphenyls should be injected on the alternate column to visualize any terphenyls present. Chloroterphenyl residues too low to be recognised in ordinary sample examination can be discovered by injections of the sample on the 1% OV-101 column.

Variations in Partitioning Among the PCB/PCT

Susan Young, Bernadette McMahon

It has been determined that the efficiency of transfer of the PCB from petroleum ether to acetonitrile decreases with increasing chlorination of the compounds. In particular, the following recoveries of Aroclor standards were obtained when a known amount of each Aroclor in 15 ml petroleum ether was partitioned into four successive 30 ml portions acetonitrile, each acetonitrile portion being backwashed with an additional 15 ml petroleum ether (FDA Pesticide Analytical Manual I (PAM I), 211.14b):

Aroclor	µg in 15 ml PE	% recovery
1221	40	94
1242	40	97
1248	40	93
1254	40	87
1260	40	78
1262	40	74
4465	20	71

Among the PCB components of any given Aroclor, those whose peaks eluted first from a DC-200 gas chromatographic column showed the highest recoveries. Percentage recovery decreased with increasing retention times (which generally increase with increasing chlorination), again suggesting that the more highly chlorinated the isomer, the poorer its transfer from petr ether to acetonitrile.

The two Aroclors made up of PCT display a similar pattern. When Aroclors 5442 and 5460 were partitioned as described above, 92 and 77% of the original 20 µg were recovered, respectively. Recoveries of individual PCT in the Aroclors were not determined, because the OV-101 GLC column for PCT does not resolve the components well enough for measurement of each.

Significance: The experiments performed in gathering this recovery data were designed to duplicate the partitioning step most often used in the analysis of fatty foods for organochlorine pesticides and/or PCB. The residue analyst should be aware that recovery through PAM I method 211 (fatty foods) will be different for different Aroclors and should avoid attributing losses completely to the Florisil or silicic acid column chromatographic steps. In addition, the pattern of PCB isomers in a given Aroclor is altered to some degree by this phenomenom, and may cause confusion to the inexperienced analyst.

Recovery of Aroclors Through Florisil Column Chromatography

New information on the behavior of Aroclors in other parts of the methodology has prompted reassessment of the elution of Aroclors from the Florisil column (PAM I 211.14d). Frequent use of pesticide residue methodology for the analysis for PCB has assured us that the chlorinated biphenyls elute in the 6% ethyl ether/petroleum ether eluate. Additional experiments have shown that Aroclors 4465, 5442, and 5460 also elute in the 6% eluate.

Recovery of Aroclors Through Silicic Acid Column Chromatography

Judith Armour, Herbert Masumoto

Masumoto (in press, JAOAC) has pointed out that some of the chlorobiphenyl isomers present in Aroclors are not eluted from the silicic acid column by petroleum ether as originally reported. Instead, the chlorobiphenyl isomers with early GLC retention times (which, generally, are those with lowest chlorination) are likely to be held on the silicic acid column and eluted from it only by the second, polar eluant. Aroclors consisting mostly of lower chlorobiphenyl isomers will therefore show low recoveries in the petroleum ether eluate.

MONS 077374

Recoveries of PCB in the following Aroclors were obtained from a column made of silicic acid and Celite (JAOAC 53, 761 (1970)) which had been previously standardized by addition of enough H_2O to effect separation of pp'DDE and Aroclor 1254:

Aroclor	Recovery
1221	essentially all in polar eluate
1242	20-35% in polar eluate
1248	less than 20% in polar eluate
1254	completely in petroleum ether
1260	completely in petroleum ether
1262	completely in petroleum ether
4465	completely in petroleum ether

The two Aroclors consisting of PCT show a similar relationship between chlorine content and elution from silicic acid. All the Aroclor 5442 added to a silicic acid column eluted in the polar eluate, while the more highly chlorinated 5460 appeared mostly in the polar eluate, but began to elute in the petroleum ether eluate.

Significance: Knowledge of the elution characteristics of the Aroclors from the silicic acid column will aid the analyst in his choice of the appropriate analytical treatment of samples containing both pesticides and PCB. If examination of the original GLC chromatogram shows the PCB to resemble an Aroclor known to be incompletely separated from pesticides by silicic acid, alternate treatment(s) can be chosen to permit quantitation of the residues.

Response of GLC Detectors to the Chlorine Content in Aroclors

Winfred Wiencke, John Roach

Experiments were performed to determine whether the responses of the electron capture and microcoulometric detectors were proportional to the chlorine content of Aroclors regardless of the nature of the compounds comprising the Aroclor. Results indicate that the response of the halogen-specific microcoulometric detector to the same amount of chlorine in different Aroclors was essentially the same. In contrast, the response of the electron capture detector to the more highly chlorinated Aroclors was disproportionately high when compared to the response of the same detector to Aroclors with lower chlorine content.

The electron capture detector that was used was first shown to give reproducible results and to be linear in response to amounts of heptachlor epoxide injected. Then, injections of six Aroclors were made and the total area of response measured for each. Calculations were performed so that responses to the different Aroclors could be compared to one another, on the basis of response/weight chlorine. The following ratios were obtained in this way:

Aroclor	Relative Response/Weight Chlorine
1221	1
1242	3
1248	4.6
1254	9.6
1260	12.7
1262	10.4

It is obvious from these data that response of the EC detector is not proportional to the amount of chlorine present in the chlorobiphenyl molecule.

In contrast, the response of the microcoulometric detector to injections of the same amounts of chlorine in each of four different Aroclors was essentially the same for each Aroclor. Because detector sensitivity changed with time, ratios of $\frac{\text{response to Aroclor X}}{\text{response to Aroclor 1242}}$ are compared, where the injection of Aroclor 1242 and the injection of the other Aroclor were made within an hour of one another. Other special precautions were also utilised to improve reproducibility; e.g., the first two injections made after cell flushing and equilibration were not considered.

The following ratios were obtained:

Relative Response

(for same weight chlorine)

Aroclor	Mean	Range (number of injections)
1242	1.00	(used as standard)
1248	0.97	0.90-1.03 (3)
1254	1.00	0.92-1.23 (5)
1262	1.10	0.86-1.26 (6)

Despite the problems encountered in dealing with the microcoulometric system, these data do present evidence that microcoulometric response is essentially proportional to the amount of chlorine present, regardless of the structure of the compounds.

Significance: The choice of Aroclor reference material against which to measure an apparent PCB residue is not always obvious. Alteration of the original Aroclor can occur in several ways and the residue may not closely resemble any of the Aroclors. The choice of reference material can significantly affect the quantitative result of the analysis when the electron capture detector is used, because of its different response to different Aroclors. The analyst should be aware of this possibility and, if possible, quantitate by microcoulometric detection when the residue identity is questionable.

H. D. Gesser, A. Chow and F. C. Davis, Chemistry Department, University of Manitoba, Winnipeg, Canada, and J. F. Uthe and J. Reinka, Fresh Water Institute, 501 University Crescent, Winnipeg, Canada

The Extraction and Recovery of Polychlorinated Biphenyls (PCB) Using Porous Polyurethane Foam

Key Words: PCB, extraction, water, polyurethane foam, pollution.

Abstract

A fast and efficient method has been developed for the extraction and concentration of PCB from water. The method uses porous polyurethane foam as the absorption column for the PCB which is eluted with acetone and hexane for G.C. analysis.

Introduction

The analysis of chlorinated pesticide residues[1] and PCB[2] in water continue to present problems. The extraction of chlorinated pesticides from water with organic solvents[3] is time consuming and can only be done with 95-100% efficiency in a laboratory. The absorption of PCB on activated carbon[4] is complete but its recovery is variable, inefficient (25-30%) and the recovered material is significantly different from the initial PCB. The reversed liquid-liquid partition filter developed by Ahling and Jensen[5] gave excellent recovery for PCB (93-100%) but is limited by relatively low flow rates ($\leq$ 65 ml/min) and requires elaborate preparation as well as vacuum or suction to achieve these filtration speeds. A recent review of the subject has been presented by Thornburg[6].

The recent paper by Bowen[7] on the absorption properties of polyurethane foams presented a new and simple method of concentrating metallic ions from dilute aqueous solution[8]. The results suggested to us that the foams have a possible absorption capability for trace organic contaminants in water and we undertook to test this for the analysis of PCB in water.

Experimental and Results

Preliminary experiments showed that polyurethane foam does extract PCB quantitatively from water. PCB was removed from the foam by Soxhlet extraction with hexane. PCB-hexane solutions were concentrated by slow evaporation to 2 ml and samples analyzed by electron capture gas chromatography. Water spiked with known quantities of PCB gave about 50% recovery for the overall method. The loss was suspected to occur through the open condenser of the Soxhlet extractor. This was verified when a second clean foam was placed in the open end of the condenser and was shown to contain a major fraction of the PCB lost.

A simpler and more efficient method therefore was developed using polyurethane foam plugs designed as culture tube stoppers (diSPo stoppers from Canlab, Winnipeg). These have a higher density (0.027 gm/cm^3) but are more porous than the commercial sheet polyurethane foam (0.022 gm/cm^3) used in preliminary work. The foam plugs (22 mm diameter and 38 mm long) were cleaned by shaking in a 1:1 hexane-acetone solution in a steel tube with a steel ball. The plugs were then pushed down a 20 mm I.D. glass chromatographic column and washed with 25 ml of ethyl alcohol to remove residual solvent and air. This was followed by careful washing with 200 ml of distilled water. The aqueous PCB solution was then passed through the column with a flow rate of about 250 ml/min without the need of pressure or vacuum. It was found that 95% of the PCB was retained by the first plug and a few percentages of PCB was retained by a second plug, thus making two plugs a necessary and sufficient column. Water was squeezed from the foam column prior to the extraction of PCB. The PCB was eluted from the polyurethane foam column with 20 ml of acetone followed by 100 ml of hexane, care being taken to wash down the walls of the glass column. It should be noted that at this stage if a significant quantity of H_2O (> 1 ml) has been co-extracted with the hexane then a separatory funnel separation of the two phases is required before the elutant is dried with about 20 g of anhydrous sodium sulfate. The dried elutant was concentrated on a rotary evaporator to 10 ml and a 5 µl sample was analyzed by G.C. The G.C. used was a Hewlat Packard Modal 5750 equipped with a ^{63}Ni electron capture detector operated at 260°C using a helium carrier gas and a 90% argon-10% methane purge gas. Pulse time was 50 microsec., range and attenuation was 1 x 128. The 6' x 1/4" glass column was packed with 2% SE-30, 3% QF-1 on 80 - 100 mesh Chromosorb W HMDS treated at a temperature of 200°C. Injection port temperature was 230°C.

The results of extraction and recovery of PCB from water by the urethane foam column are summarized in Table 1 where quantitative calculations are based on the peak height of 13 resolved peaks, with the average and mean deviation for each analysis given.

TABLE 1

The Extraction and Recovery of PCB From 1 Liter of Water

PCB added	PCB analyzed	% recovery
20 µg.	19.6	98±6
20 µg.	19.0	95±4
20 µg.	18.6	93±14
20 µg.	19.0	95±3
20 µg.	18.2	91±7

Similar results were obtained with 2.0 µg/liter of PCB in water. It was also shown that PCB is not washed out of the foam by water. A

complete analysis can be done within one hour. The results clearly indicate the method to be both fast and efficient. The extraction can be performed at the water sampling site and the foam plug (if column wall interference is avoided) or the acetone-hexane solution can be returned to the laboratory for concentration and analysis.

Discussion

The limits of detection and analysis of PCB in water is presumably governed by the amount of water chromatographed through the foam column. The polyurethane foam is not specific to PCB and it will absorb organochlorinated pesticides as well and a complex mixture would require some separation[9,10] prior to G.C. analysis.

The application of this method to inorganic ions will be reported shortly.

Acknowledgements

Grateful acknowledgement is made for partial support from the National Research Council of Canada and Manitoba Scientists to Combat Pollution through an Opportunities for Youth Program grant.

References

1. A. Bevenue, T. W. Kelley and J. W. Hylin, J. Chromatog. 54, 71 (1971).
2. G. D. Veith and G. F. Lee, Water Res. 4, 265 (1970).
3. L. Kahn and C. H. Wayman, Anal. Chem. 36, 1340 (1964).
4. J. J. Lichtenberg, PCB Newsletter, Aug. 20 (1970).
5. B. Ahling and S. Jansen, Anal. Chem. 42, 1483 (1970).
6. W. Thornburg, Anal. Chem. 43, 145R (1971).
7. H. J. M. Bowan, J. Chem. Soc., 1082 (1970).
8. P. Schiller and G. B. Cook, Anal. Chim. Acta 54, 364 (1971).
9. J. A. Armour and J. A. Burke, J. Ass. Offic. Anal. Chem. 53, 761 (1970).
10. N. V. Fenringer and J. E. Westfall, J. Chromatog. 57, 397 (1971).

Ronald G. Webb, Research Chemist, EPA, Southeast Water Laboratory, Athens, Georgia 30601

Chemical Identification of PCB Isomers

A paper on "Identities of Polychlorinated Biphenyl (PCB) Isomers in Aroclors" by R. G. Webb and Ann C. McCall has been accepted for publication in the July issue of the Journal of the Association of Official Analytical Chemists. Preprints are available.

Another excellent paper in this area is by D. Sissons and D. Welti, Journal of Chromatography, 60, 15 (1971).

Two pure PCB isomers found in Aroclors 1242-1254 are available as 10 mg samples. The isomers are 2,5,2',5'-tetrachlorobiphenyl and 2,4,5,2',5'-pentachlorobiphenyl.

MCNS 077379

We are working on the preparation of a series of Aroclor solutions to be used as quantitative standards, particularly for electron-capture GC analysis. We hope to provide solutions in which the amount in each major GC peak is known as well as the total amount present together with the response factor of each peak relative to p,p'-DDE. Our target date is May 1.

ENVIRONMENTAL OCCURRENCE AND FATE

George R. Harvey, H. P. Miklas, V. T. Bowen, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Chlorinated Hydrocarbon Pollutants in the Atlantic Ocean

Summary of Investigations, 1971

We are engaged, in close cooperation with the Department of Biology, in determining the concentrations of polychlorinated biphenyls (PCB) and members of the DDT family in organisms collected from the open Atlantic Ocean, North and South. This is the first study of its kind in the high seas and has furnished us with new insights into transport mechanisms and food chains.

The data strongly indicate that the atmosphere provides the major path for delivery of both DDT and PCB to the ocean. The North Atlantic plankton show almost uniformly high levels (100-900 ppm) in their fat. Thus, the quantities of these pollutants delivered to the ocean by the atmosphere obliterate any concentration gradient from continental run-off. Secondly, there is very little difference in concentrations of these pollutants between the North and South Atlantic. Considering the smaller land surface of the southern hemisphere, the low level of industrialisation and the lack of North to South circulation of Atlantic surface waters, the South Atlantic pollution must be transported there by global winds from the northern hemisphere.

Our data appear to offer no support for the widely held view that concentrations of chlorinated hydrocarbons are magnified along marine food chains. Many prey-predator couples (flying fish-plankton, Arctic Cod - Atlantic Cod, etc.) reveal concentrations just the reverse of what would be expected from biomagnification. Different species which have similar diets and collected in the same nets show widely different concentrations of DDT and PCB. We believe that the levels found within an organism are a result of the partitioning of the DDT and PCB between the water and the body lipids. The lipids of each species will have a definite capacity for these chemicals regardless of its position in the food chain. Air breathing animals must depend on excretion and metabolism, which are apparently less efficient than equilibration with water, and accumulate these pollutants. This discovery would have been extremely difficult to make in coastal waters where the organisms are subjected to sporadic and high doses of pollutants from spills, floods and dumping operations. The open-ocean is remote enough so as not to be affected by daily and local inputs.

Finally, PCB formulations must be transported to the ocean by discreet aerosol masses which do not mix with similar formulations during their atmospheric history. This phenomenon is not shared by DDT which is largely transported on atmospheric particulate matter. This must be a consequence of the different modes of injection into the atmosphere: DDT by slow evaporation from soil and PCB by distilling from incinerators, dump fires and explosions (aircraft crashes).

MONS 077382

We are continuing to explore these phenomena and will soon study the mechanisms of uptake and transfer of chlorinated hydrocarbons in the open-sea.

These studies are being supported by the National Science Foundation under Grant GX 28334 and by the U. S. Atomic Energy Commission under Contract AT(30-1)-3563.

John L. Hesse, Michigan Water Resources Commission, Lansing, Michigan, December 17, 1971

PCB's in Michigan Waters

The following is a brief summary of the Michigan Water Resources Commission PCB monitoring results during 1971.

Analyses of 98 water samples (30-hour composites) from 30 major tributaries to the Great Lakes indicate that PCB contamination of surface waters is widespread with 71 percent of all samples having detectable (greater than 10 ppt) concentrations. Concentrations greater than 100 ppt have been detected in seven streams, all located in the industrialized southeastern portion of the lower peninsula. The Saginaw River has consistently had the highest concentrations with as much as 2,900 ppt on one date.

Sampling of Great Lakes water intakes has shown much lower PCB concentrations than in tributary streams. Only 3 of 36 samples contained detectable concentrations with a high of 300 ppt.

Limited sampling of groundwater supplies in the southern lower peninsula has indicated that groundwater has not been significantly contaminated with PCB's. One of 4 samples contained 19 ppt while the other 3 were less than 10 ppt.

Analyses of surface water runoff from sanitary and industrial landfills in Michigan suggest that these are a minor source of environmental contamination. Five of the 9 samples collected had PCB concentrations ranging from 40 to 300 ppt while the others contained less than 10 ppt.

Discharges from municipal wastewater treatment facilities appear to be a very significant source of PCB losses to the waters of the state. Twenty-eight of 32 WWTP discharges sampled contained greater than 100 ppt PCB's. Eight had concentrations in excess of 1,000 ppt with a high of 336,000 ppt. Investigations have been initiated which should identify the major sources of input to the municipal systems with the greatest contamination.

Discharges from a cross-section of industries throughout Michigan were sampled for PCB concentrations. PCB losses from industries is apparently very widespread. Thirty of 58 industrial discharges tested had PCB concentrations greater than 100 ppt. Eleven industries had discharges containing PCB's in excess of 1,000 ppt.

Because the Saginaw River was identified as having the highest PCB concentrations of any major tributary to the Great Lakes within Michigan, an intensive survey effort was conducted to isolate the major sources of PCB contamination in that basin. Water samples collected at key locations ranging from each of the four major tributaries of the Saginaw down to the mouth of the river indicated that the primary increase in PCB concentrations occurs in a short zone in the immediate vicinity of Bay City and Essexville. More intensive sampling in that zone revealed two major sources of PCB losses to the river. Actions were taken toward elimination of these sources of PCB contamination.

James J. Lichtenberg, Group Leader Pesticides Identification, Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268

In anticipation of a possible probe into the historical occurrence of PCB's in surface waters, stored carbon adsorption sample extracts (CCE) from three selected sites were analysed. The samples were cleaned up and separated on a microcolumn of Davison 950 silica gel according to the procedure of Leoni [Journal of Chromatography, 62, 63 (1971)] prior to gas chromatographic determination. The results show an estimated 1 to 2 µg/l of PCB's in one sample. The chromatogram suggested a probable mixture of Aroclors 1242, 1248, and 1254. A trace of what appeared to be Aroclor 1232 was noted in another sample. The third sample showed no Aroclor characteristics. These positive occurrences are significant in light of the fact that they occurred in samples collected in mid-1966.

Effective procedures for clean-up and separation of the Aroclors from pesticides and other interfering materials are required for adequate analysis of CCE. Of the published procedures that we have tested, the Leoni procedure has proven the most useful for this purpose.

Ronald Parejko, Department of Biology, Northern Michigan University, Marquette, Michigan 49855

At Northern Michigan University, Marquette, Michigan, I am establishing a chlorinated hydrocarbon pesticides and PCB analysis laboratory. This laboratory will have its center of interest, at least initially, focused upon the quantification of PCB's in lake trout taken from Lake Superior.

The lab is currently in the primary stage of setting-up and as yet no data has been accumulated. Funding for the installation of the laboratory has been provided by the Office of Water Resources Research, U. S. Department of the Interior.

MONS 077384

TOXICITY AND OTHER PHYSIOLOGICAL EFFECTS

MONS 077385

Pete Benville, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Toxicity of Aroclors® to Aquatic Organisms

Fish: Tests to determine the chronic toxicity of Aroclors® to fish are continuing. Aroclors® 1242, 1248, and 1254 were about equally toxic to yellow perch (30-day TL50 = 72 to 92 µg/l), while Aroclor® 1260 was less than half as toxic.

Herman O. Sanders, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Invertebrates

Preliminary static bioassays with individual PCB isomers were begun using scud (Gammarus pseudolimnaeus). These organisms were selected as the amounts of pure isomers available did not permit bioassays using larger organisms.

No. Cl	PCB Component	TL50 (mg/l) 24 hr	96 hr
2	2,4'-dichlorobiphenyl	0.15	0.12
2	4,4'-dichlorobiphenyl	0.16	0.10
3	2,3,4'-trichlorobiphenyl	0.10	0.07
5	2,4,5,2',5'-pentachlorobiphenyl	0.43	0.21
6	2,4,6,2',4',6'-hexachlorobiphenyl	0.20	0.15
4	mixture of 3, decachlorobiphenyls	0.30	0.11

Foster L. Mayer, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Chronic Toxicology of PCBs to Fish

TL50 values (5-30 days) from continuous flow exposures of Aroclors® 1242, 1248, 1254, and 1260 to three species of fish have been determined. Comparative toxicity data for DDT was also determined.

Compound	Species	TL50[1] (µg/l) for 5-30 days					
		5	10	15	20	25	30
DDT	RBT[2]	4.22	1.39	0.51	0.41	0.22	-
	BG[3]	1.18	1.43	1.63	1.27	0.59	0.35
	CCF[4]	>1.0	1.54	2.33	1.32	0.87	0.57
1242	RBT	67	48	18	10	12	-
	BG	125	125	164	125	120	84
	CCF	>100	125	219	150	132	87
1248	RBT	54	38	16	6.4	3.4	-
	BG	136	115	111	106	100	78
	CCF	>100	121	121	115	104	75
1254	RBT	142	160	64	39	27	-
	BG	>200	>200	303	260	239	177
	CCF	>200	303	286	293	181	139
1260	RBT	232	326	143	78	49	51
	BG	>400	>400	>400	>400	>400	>400
	CCF	>400	535	482	512	465	433

[1] Temperature, 17°C

[2] Rainbow trout

[3] Bluegills

[4] Channel Catfish

These data compare favorably with our previously determined TL50 values at a comparable exposure period. However, these TL50 values are somewhat higher than the previous values. Dietary protein levels were increased during the two test periods and may have decreased the PCB toxicity during this extended exposure. Toxicity appears to be inversely related to chlorine content and at 30 days had not become independent of exposure time.

George Yip and James D. Link, U. S. Food and Drug Administration, Washington, D.C. 20204

PCB Metabolism

Aroclor 1254 residues were determined on organs of rats which had been given single daily doses of 300 mg/kg Aroclor 1254 for 5 days or 15 mg/kg for 14 days. Livers, kidneys, fat, blood, and urine were analyzed by electron-capture GC. Except for differences in amounts found, all samples had similar type chromatograms showing major losses of early eluters and more significantly, a complete loss of one major peak. Using a standard supplied by Dr. R. G. Webb, this peak has been tentatively identified as 2,3,6,3',4'-pentachlorobiphenyl by GLC and IR. Quantities of this component will be obtained for further in vivo and in vitro studies to determine its fate in biological systems.

S. Bailey, P. J. Bunyan, and J. M. J. Page, Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Hook Rise South, Tolworth, Surbiton, Surrey

Investigations are now under way into the effects produced by individual polychlorinated biphenyl isomers following low level chronic feeding to Japanese quail. Various parameters of microsomal activity are being measured and an attempt will be made to correlate these with tissue residue levels. Excreta are being monitored for metabolic transformation products. It is hoped that an overall picture will emerge relating these measurements to the extent and pattern of chlorination.

L. S. Merkens and W. B. Kintner, Departments of Pharmacology and Physiology, Upstate Medical Center, SUNY, Syracuse, New York 13210

Two types of studies were done with DDT and Aroclor 1221 (provided by Monsanto Co.) in marine adapted killifish (Fundulus heteroclitus). Acute toxicity and changes in serum osmolality were measured. Both tests were done using a simple static system. In each experiment 10 fish, each 5 g, were maintained in 2 liters of sea water at 15 C with 0.1% ethanol. The experimental compounds were first dissolved in 2 ml of ethanol before added to the sea water. Aroclor 1221 at 75 ppm (theoretical concentration in the sea water) was less toxic than DDT by a factor of about 100. It

demonstrated roughly equivalent lethality (24 hours) as 0.25 ppm DDT. Exposure of these same levels (75 ppm Aroclor 1221 and 0.25 ppm DDT) for 6 hours increased serum milliosmolality (determined by freezing point depression) from 365 ± 3 (SE) (6 samples each from 3-5 ethanol control fish) to 396 ± 7 (9) and 389 ± 9 (8), respectively ($p<0.05$). A more complete description of methods and data will appear in Environmental Health Perspectives.

D. R. Nimmo, D. J. Hansen, N. R. Cooley, and J. A. Couch, Environmental Protection Agency, Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida 32561

The Environmental Protection Agency Laboratory at Gulf Breeze, Florida, an associate laboratory of the National Environmental Research Center, Corvallis, Oregon, is continuing to study the effects of polychlorinated biphenyls on estuarine organisms. We are now completing many experiments reported in the last newsletter and beginning new studies.

Studies have been completed on the effect of Aroclor 1248 on growth of populations of the ciliate protozoan, Tetrahymena pyriformis W, employing as criteria of effect the alteration of growth rate during exponential growth and of population density at 96 hours, when control populations are maximal. The ability of this ciliate to accumulate PCB from the medium and concentrate it was investigated. Similar studies of Aroclor 1260 are nearing completion.

The relationship of salinity of test water and toxicity of Aroclor 1254 to longnose killifish, Fundulus similis, is being investigated. As fish die, they are dissected and samples of individual tissues as well as composite samples of whole fish are analyzed for Aroclor 1254. After half of the fish die, the survivors are also dissected for residue analysis. Results are incomplete.

We have studied the effects of 0.1, 1.0, and 10.0 ppb of Aroclor 1254 on setting and growth of animals in sand-filled aquaria during a four-month exposure. Most animals have been identified and enumerated. Species diversity indices have been computed, but statistical analysis of the data is incomplete.

The ability of animals to avoid Aroclor is being tested by allowing them to move into water containing Aroclor or into Aroclor-free-water. Experiments using pink shrimp, Penaeus duorarum, and mosquito fish, Gambusia affinis, have been completed. Use of other crustaceans and fishes is being considered.

A crystalloidal material has been found in the nuclei of hepatopancreatic cells of shrimp exposed to 5 ppb Aroclor 1254 for long periods of time. Also, liver necrosis has been found in spot, Leiostomus xanthurus, similarly exposed to this PCB.

We have found the tolerance limit of 50% of a population of estuarine grass shrimp, Palaemonetes pugio, to be between 4.0 and 12.5 ppb Aroclor 1254. In this 16-day experiment, we found correlations between concentration of this material in the water and mortality. Preliminary tests showed that Palaemonetes kadiakensis, the fresh-water grass shrimp found in this area, was much less susceptible to this PCB than P. pugio, the local estuarine grass shrimp.

Dave Hansen reported that a newly identified source of contamination of Aroclor 1254 in bioassay water to be Teel submersible pumps, No. 1P597, that were used to supply water to a constant-head box. At times, concentrations in the water exceeded 0.2 ppb (µg/l). Residue analyses performed on various portions of the pump revealed that the grease used to lubricate the shaft was about 0.37 percent polychlorinated biphenyl.

Therefore, this pump should be used only if the grease is replaced or in experiments in which presence of this contaminant would not alter results.

Alan V. Nebeker, Research Aquatic Biologist, Western Fish Toxicology Laboratory, NWQL, U. S. Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, Oregon 97330

Toxicity of Polychlorinated Biphenyls (PCB's) to Fish and Other Aquatic Life

Final Report

Summary of Findings (Abstract)

Aroclor 1248 was the most toxic of the eight Aroclors tested to Daphnia magna in static tests, with 16 percent impairment of reproduction occurring at 16 µg/liter. Aroclor 1254 was most toxic under continuous-flow conditions with 16 percent reproductive impairment at 0.48 µg/liter. The Aroclors were much more toxic under continuous-flow than static conditions, with 16 percent impairment of reproduction by Aroclor 1248 occurring at 1.0 µg/liter.

Calculated 96-hour LC50 values for newly-hatched fathead minnows were 7.7 µg/l for Aroclor 1254 and 15 µg/l for 1242. Three-month-old fatheads had a 96-hour LC50 of ca. 300 µg/l for 1242. Reproduction occurred at and below 1.8 µg/l 1254 and at and below 5.4 µg/l 1242. Polychlorinated biphenyls were acutely toxic but exhibited much greater chronic toxicity at very low levels, due to their cumulative nature. Newly hatched young were the most sensitive life stage. Young fathead growth was also affected above 2.2 µg/l 1248 and none survived above 5.1 µg/l after 30 days.

Young flagfish, Jordanella floridae, did not survive above 5.1 µg/l 1248 and did not grow well above 2.2 µg/l.

MONS 077390

Ninety-six hour LC50 values for Aroclor 1242 and 1248 with Gammarus pseudolimnaeus were 73 and 29 µg/liter. Survival after 30 days was 53 percent at 8.7 µg/l 1242 and 52 percent at 5.1 µg/l 1248. Threshold values were unobtainable as deaths continued to occur until end of test, indicating the cumulative chronic toxicity of PCB's. Good reproduction and survival of young occurred at 2.8 µg/l 1242 and 2.2 µg/l 1248.

Abundant adult emergence of the midge Tanytarsus dissimilis did not occur above 5 µg/l 1248 or 3.5 µg/l 1254. The calculated 3-week LC50 (50% reduction based on control as 100%) for Aroclor 1254 was .65 µg/l for larvae and .45 µg/l for pupae.

Application factors of 0.10 for 1242 and 0.15 for 1248 were calculated for the PCB's and Gammarus. Application factors of 0.2 for 1254 and 0.16 for 1242 were calculated for fathead minnows using newly hatched fish to obtain the 96-hour LC50.