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# POLYCHLORINATED BIPHENYL RETENTION TIME STANDARDS OBTAINED BY CHEMICAL DECHLORINATION OF POLYCHLORINATED BIPHENYL ISOMERS

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## SUMMARY

Polychlorinated biphenyls (PCBs) were obtained by the partial catalytic dechlorination of individual PCBs having up to six chlorine atoms. The dechlorination was accomplished with sodium borohydride and nickel boride catalysis generated *in situ*. All of the expected PCB congeners were observed when dechlorinating PCBs with four or less chlorine atoms. The mixtures were separated by gas chromatography on a methyl silicone capillary column. Identification of individual congeners was made by comparison of retention indices (RI) to literature, experimental and estimated values and by cross comparison of peaks generated from different parent PCBs. Various methods of estimating RIs were evaluated.

The effects of varying concentration and reaction time were studied along with the relative reactivity of chlorines in different positions. The partially dechlorinated PCB mixtures are particularly useful as retention time standards for PCBs with less than four chlorine atoms.

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## INTRODUCTION

Polychlorinated biphenyls (PCBs) were used in diverse applications such as electrical capacitors and transformers, hydraulic systems and carbonless copy paper[1]. The resistance of PCBs to degradation and their accumulation in animal tissue make them an object of environmental concern and the subject of extensive research. The 209 theoretically possible PCB congeners make the separation and identification of individual members of PCB mixtures difficult. The publication of high resolution gas chromatographic (GC) data on commercial PCB mixtures[2–6] has helped in the identification of individual PCBs. These studies, however, are of limited value if different GC liquid phases, detectors or temperature conditions are used. The identification of PCBs that have not been characterized by GC, such as photodegradation products, can be difficult to achieve. GC–mass spectrometry (MS) can determine the number of chlorine atoms on a PCB molecule but provides almost no information on the chlorine substitution pattern[7]. The use of calculated retention indices (RI) is extremely helpful in limiting the number of possible congeners to be considered in an identification. The use of PCB standards provides more precise identification, but the use of standards to determine exact retention times is hampered by the high cost or their need to be synthesized. To overcome these difficulties, the synthesis of PCB mixtures that would be suitable as retention time standards was carried out by the partial dechlorination of individual PCB congeners. The identification of members of the resulting mixtures was facilitated by the limited number of products formed from a given PCB. Calculated retention indices were used to obtain tentative identifications of the peaks. Cross comparisons of different PCB congener's products were used to confirm identifications.

Zimmerli's quantitative determination of trace amounts of halogenated environmental contaminants with deactivated palladium catalyst to reduce them to their dehalogenated parent compounds has demonstrated the value of dechlorination techniques[8].

## EXPERIMENTAL

A Hewlett-Packard 5840A gas chromatograph equipped with a 10 m × 0.21 mm I.D. fused-silica capillary column was used in the splitless mode. The dimethyl silicone fluid coated capillary column, supplied by Hewlett-Packard, was temperature-programmed at a rate of 5°C/min from 60°C tot 210°C. Helium was used as the carrier gas at a flow-rate of 0.5 ml/min and nitrogen, at a flow-rate of 30 ml/min, for the make-up gas. The injection port was kept at 200°C and the flame ionization detector at 250°C.

The 2,3′,4′,5-tetrachlorobiphenyl was obtained from Analabs (North Haven, CT, U.S.A.). The 2-; 3-; 4-; 2,2′-; 3,3′-; 4,4′-; 2′,3,4-; 2,4′,5-; 2,2′,4,4′-; 2,2′,5,5′-; 2,2′,4′,5-; 2,3′,4′,5-; 2,3,4,5,6-; 2,2′,3,4,6- and 2,2′,3,3′,6,6′-PCBs were from Ultra Scientific (Hope, RI, U.S.A.).

The partial dechlorination of the PCB congeners was carried out by a modification of the method of Dennis *et al.*[9], developed for the destruction of Aroclors. A volume of 200 $\mu$l of a 1 *M* aqueous solution of sodium borohydride was added to a 100 × 13 mm test tube equipped with a PTFE cap containing a mixture of 100 $\mu$g of a PCB dissolved in 1 ml of isopropanol, 0.4 ml of water and 5 $\mu$l of an aqueous 2 *M* nickel chloride solution. The kinetic experiments were conducted by dripping the sodium borohydride solution down the side of the test tube over a period of 10 to 15 sec. Fifteen seconds after starting the addition of the sodium borohydride the tube was shaken and the reaction maintained at 25°C. Any hexane solvent used to transfer PCB to the test tube should be evaporated and its vapor removed from the tube with a stream of nitrogen as it will inhibit the reaction.

The reaction was stopped by adding 4 ml of water and the mixture extracted with three 1-ml portions of hexane. The combined extracts were washed with 1 ml of water to which two drops of 10% sulphuric acid had been added. The extract was passed through a 22.5 × 0.6 cm I.D. pasteur pipette filled to a height of about 5 cm with anhydrous sodium sulphate. The test tube was rinsed twice with 1-ml portions of hexane which were added to the column. The eluate collected from the column was evaporated to 1 ml in a stream of nitrogen. Particular care should be used when handling 3,3′,4,4′-; 3,3′,4,4′,5- and 3,3′,4,4′,5,5′-PCBs as these have been shown to be strong inducers of cytochrome enzymes[10,15].

The retention indices were calculated from temperature programmed data by the following formula:

$$\mathrm{RI} = 100\,N + 100\,\frac{T_{\mathrm{PCB}} - T_1}{T_2 - T_1}$$

Where $T_{\mathrm{PCB}}$ is the retention time of PCB, $T_1$ the retention time of the $n$-alkane eluting before the PCB, $T_2$ the retention time of the next $n$-alkane in the series which elutes after the PCB, and $N$ the number of carbons in the lower alkane[11]. The retention time print-out was in hundredths of a minute. This corresponds to 0.4 index units.

## RESULTS AND DISCUSSION

The partial dechlorination of individual PCB congeners containing fewer than five chlorine atoms yielded the complete set of expected products. The partial dechlorination of a tetrachlorobiphenyl of low symmetry produces as many as 14 products. The reactions of the penta- and hexachlorinated PCBs produced only a fraction of the tetra- and pentachlorinated products in significant yield. A chromatogram of partially dechlorinated 2,2′,5,5′-tetrachlorobiphenyl is shown in Fig. 1.

### *Identification of products*

The products were identified by comparing the retention indices of the products with indices derived from other sources. The cross comparison of reaction products, produced from various PCB congeners, was also used to identify peaks and was especially useful in establishing the identity of peaks possessing similiar indices. In obtaining indices to compare to the product indices, six methods were tried. Table I summarizes the six methods. To compare the effectiveness of the six methods, nine PCBs were selected on the basis of their applicability to the six methods. The PCBs ranged from dichloro- to tetrachlorobiphenyls.

The first three methods are based on the principle that each half of the biphenyl

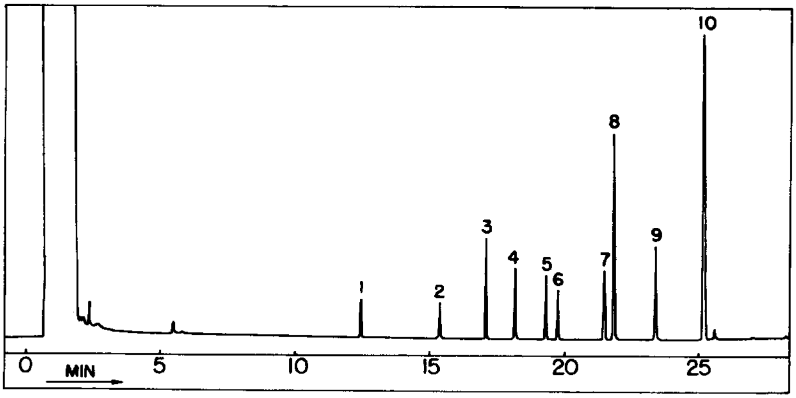

Fig. 1. Separation of the dechlorination products of 2,2′,5,5′-tetrachlorobiphenyl on a capillary column coated with dimethyl silicone. See Experimental section for details. Peak numbers and structures: 1, biphenyl; 2 and 3 correspond to 2- and 3-chlorobiphenyl; 4–7 correspond to 2,2′-; 2,5-; 2,3′- and 3,3′-dichlorobiphenyl; 8–10 correspond to 2,2′,5-; 2,3′,5- and 2,2′,5,5′-tetrachlorobiphenyl.

TABLE I

METHODS USED TO ESTIMATE PCB INDICES

All columns used dimethyl silicone as a liquid phase. See text for additional details.

| *Method* | *Data source* | *Nature of data* | *Correction method* | *Column dimensions (m × mm I.D.)* | *Temperature (°C)* |
|---|---|---|---|---|---|
| I | Albro *et al.*[12] | ½(RI) | Additivity constants | 0.3 × 2 | 200 |
| II | Albro *et al.*[12] | ½(RI) | Linear regression | 0.3 × 2 | 200 |
| III | Present work | ½(RI) | – | 10 × 0.21 | 60–210 (5°C/min) |
| IV | Onuska and Comba[13] | RI | Additivity constants | 20 × 0.26 (WCOT) | 100–200 (2°C/min) |
| V | Onuska and Comba[13] | RI | Linear regression | 20 × 0.26 (WCOT) | 100–200 (2°C/min) |
| VI | Present work | RI | – | 10 × 0.21 | 60–210 (5°C/min) |

ring makes a simple additive contribution [½(RI)] to the retention index of a PCB[3,12]. Method II and V involved a linear regression between literature data and the product indices.

In method I the data of Albro *et al.*[12], on the contributions of chlorinated rings to the retention index of PCBs, were used to calculate a set of indices for all of the possible PCB products. These data were obtained on a (300 × 0.2 cm I.D.) column packed with 10% OV-101 on 80–100 mesh Chromosorb W HP at 200°C. A simple additivity constant was used to provide a correction between Albro's isothermal data and my temperature programmed data. The mean difference between Albro's uncorrected indices and the indices of nine of the products produced by partial dechlorination was 30.8 index units. Subtracting this value from Albro's data resulted in a standard deviation (S.D.) of 6.2 index units between Albro's corrected data and my experimental indices (Table II).

TABLE II

COMPARISON OF METHODS USED TO ESTIMATE RIs

| *PCB structure* | *Experimental RIs* | *Error of estimated RIs (RI units)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Method* | | | | | |
| | | *I* | *II* | *III* | *IV* | *V* | *VI* |
| 2,5 | 1629.4 | – 2.2 | –6.5 | –4.8 | 12.5 | 2.3 | –0.7 |
| 2,3′ | 1648.9 | 8.3 | 4.9 | 4.0 | 11.0 | 2.3 | 0.0 |
| 2,4′ | 1660.0 | 7.2 | 4.2 | 2.6 | 2.9 | –5.5 | 0.0 |
| 3,3′ | 1725.4 | – 2.2 | –3.4 | 0.0 | 3.5 | 0.8 | – |
| 2,2′,5 | 1742.1 | – 1.9 | –2.5 | –1.7 | 1.8 | 0.4 | –0.4 |
| 2,2′,3 | 1775.7 | 2.5 | 3.1 | 0.4 | – 2.8 | –1.7 | –0.5 |
| 2′,3,4 | 1848.6 | 3.6 | 6.6 | – | – 3.7 | 3.6 | –0.3 |
| 2,2′,5,5′ | 1900.4 | –11.4 | –6.9 | 0.0 | –12.5 | –1.5 | –0.4 |
| 2,2′,4,4′ | 1913.6 | – 4.4 | 0.5 | 0.0 | –12.7 | –0.6 | 0.2 |
| Standard deviation | | 6.2 | 5.0 | 3.5 | 8.9 | 2.7 | 0.3 |
| Correlation coefficient | | – | 0.9989 | – | – | 0.9997 | – |
| Mean error | | – 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | –0.3 |

Method II is a refinement of method I in which a linear regression between Albro's data and data derived from the dechlorination products was done on the nine PCBs compared in Method I. The correlation coefficient of the data sets was 0.9989. The results in Table II show a difference of 5.0 index units between the experimental data and the data corrected by the regression formula. Only a small improvement was achieved by using a linear regression, due to high degree of scatter in the data. The regression formula had a slope of 1.033. When Method I or II is used it is best to leave out mono- and dichlorophenyl data when determining the correction constants since their indices differ from the predicted values by approximately 15 index units.

In Method III the ½(RI) contribution of each ring was calculated using the retention index data of the dechlorination experiments. This was achieved by dividing the index values of the symmetrical PCBs by two. By subtracting the resulting ½(RI)s from the indices of the unsymmetrical PCBs many additional ½(RI)s were obtained.

In calculating ½(RI)s from temperature programmed data no correction was made for the different temperatures at which each ½(RI) was obtained. The retention index temperature coefficients are fairly independent of temperature as well as being roughly equal for most of the PCBs[14]. This, combined with the fact that the elution temperature of the peak whose retention index is being calculated will be close to half way between the elution temperatures of the PCBs from which it was calculated, results in most of the temperature correction being cancelled. There is a similar cancellation when ½(RI)s derived indirectly from the unsymmetrical PCBs are used.

For purposes of comparing the accuracy of Method III to the other methods, the symmetrical PCBs in Table II were deleted from the statistical calculations. By the nature of their index evaluation, their error would be zero. The differences between the experimental and the calculated data for the remaining seven PCBs is shown in Table II to be 3.5 index units.

It has been noted in the literature[2,3] that data derived from ½(RI)s have relatively large errors when calculated for some of the more highly chlorinated PCBs. In particular, RIs of PCBs that contain a high degree of chlorine substitution in the ortho positions, or contain rings having a high dipole moment such as a 2,3-dichloro ring, should be calculated with these problems in mind. The preferred retention index for a PCB containing a 2,2′,6- or 2,2′,6,6′-substitution pattern is one that has been derived from PCBs with similar substitution patterns.

Method IV is a comparison between the PCB retention index data of Onuska and Comba[13] and the nine experimental PCB indices of Table II. The data of Onuska and Comba[13] were obtained on a 20 m × 0.26 mm I.D. SP-2100 WCOT Pyrex capillary column programmed from 100 to 200°C at 2°C/min with a 3-min initial hold. The average difference of 0.1 index units between the two data sets was used as a correction constant by subtracting it from Onuska and Comba's data. As can be seen in Table II, the differences between the Onuska corrected data and the PCB retention index standard data have a S.D. of 8.9 index units. A progressive change in the size and direction of the error can be seen in Table II as the retention indices increase. To correct for this progressive change, a linear regression was performed on the uncorrected data of Onuska and Comba[13] and the indices of the dechlorination products. The results are shown under Method V of Table II. The S.D. of the difference between the indices calculated from the regression formula and indices of the PCB standards is only 2.7 index units. The regression formula had a slope of 1.090 and a correlation coefficient of 0.9997. The large improvement in accuracy is gained in using method V compared to method IV. This gain is greater than that seen in method I and II because of the small degree of data scatter in methods IV and V.

In Method VI a GC chromatogram of Aroclor 1016 was obtained under the same conditions as used for the dechlorination products in Table II. The S.D. and mean error between the two sets of data were 0.3 and −0.3 index units respectively. Given the errors in determining retention times and the presence of unresolved components in some Arochlor 1016 peaks, this degree of error is reasonable. The insignificant mean error (Table II) observed for the other methods is due to the correction procedures used, except for Method III which has a fortuitously low mean error.

The GC peaks of Aroclor 1016 were identified by comparison to the high resolution chromatograms of Ballschmiter and Zell[2]. After establishing the identity of a few peaks, the remaining peaks were easily identified, in spite of the fact that electron-capture detection data were being compared to flame ionization detection data. However, when identifying Aroclor peaks by comparison to literature chromatograms, a number of points must be kept in mind. Close lying peaks can change

TABLE III

THE RETENTION INDICES AND STRUCTURES OF THE TETRACLOROBIPHENYL DECHLORATION PRODUCTS

Parent PCBs: 1 = 2,2′,4,4′; 2 = 2,2′,4′,5; 3 = 2,2′,5,5′; 4 = 2,3′,4′,5.

| *RI* | *Structure* | *Parent PCB* |
|---|---|---|
| 1349 | 0 | 1–4 |
| 1465 | 2 | 1–4 |
| 1535 | 3 | 2–4 |
| 1542 | 4 | 1,2,4 |
| 1580 | 2,2′ | 1–3 |
| 1629 | 2,5 | 2–4 |
| 1630 | 2,4 | 1,2,4 |
| 1649 | 2,3′ | 2–4 |
| 1660 | 2,4′ | 1,2,4 |
| 1725 | 3,3′ | 3,4 |
| 1737 | 3,4′ | 2,4 |
| 1742 | 2,2′,5 | 2,3 |
| 1746* | 4,4′ | 1 |
| 1746* | 2,2′,4 | 1,2 |
| 1814 | 2,3′,5 | 3,4 |
| 1817 | 2,3′,4 | 2 |
| 1829 | 2,4′,5 | 2,4 |
| 1831 | 2,4,4′ | 1,2 |
| 1849 | 2′,3,4 | 4 |
| 1900 | 2,2′,5,5′ | 3 |
| 1909 | 2,2′,4′,5 | 2 |
| 1914 | 2,2′,4,4′ | 1 |
| 1932 | 3,3′,4 | 4 |
| 2019 | 2,3′,4′,5 | 4 |

* Merged with neighboring peaks.

their relative position when obtained under different temperature conditions[3]. Stalling *et al.*[15] have observed the reversal of a pair of GC peaks as the result of column aging when using Apiezon L. Changes in column surface activity and in the composition of the liquid phase may be responsible for such changes. Whether or not the Apiezon L is purified and the type of purification used may also affect retention indices.

Batch to batch variations in the composition of commercial PCB mixtures can also be troublesome. A particularly striking change in the distribution of congeners in Aroclor 1254 occurred in the 1970s as Monsanto changed from a batch to a continuous process[4].

In general, Methods V and VI are useful for identifying the congeners present in commercial PCB mixtures, if the same GC liquid phases as those cited in the literature are used. If a different liquid phase has to be used to resolve a pair of peaks, or if a PCB is not present in a commercial mixture, Methods I–III have sufficient accuracy to identify the components of the dechlorination mixtures, when used in conjunction with the cross comparison of suitable dechlorination mixtures. Table III shows the dechlorination products, the parent PCBs and retention indices that can be obtained from only four tetrachlorobiphenyls.

### *Reactivity of PCBs*

The relative reactivity of each position was measured. The order of reactivity was *para*>*meta*>*ortho* for both the mono- and dichlorobiphenyls (Table IV). The dichlorobiphenyls were also more reactive than the monochlorobiphenyls. Comparing the peak abundance of the reaction products of 2′,3,4- and 2,4′,5-trichlorobiphenyls (Table V), some relative reaction rates are different than the above data would indicate. Even a rough estimate of concentrations shows that the 2- position in the 2′,3,4-trichlorobiphenyl products and the 4-position in the 2,4′,5-trichlorobiphenyl products are present in the predominant peaks. The observation that the

TABLE IV

COMPOSITION AND CONCENTRATION OF REACTANTS AND PERCENT OF STARTING MATERIAL AT END OF REACTION

Congeners were run simultaneously in groups of three. The reactions were reacted for 1 min at 25°C. See Experimental section for additional detail.

| *PCB congener* | *Weight added (μg)* | *Percent remaining (%)* |
|---|---|---|
| 2 | 30 | 78 |
| 3 | 30 | 76 |
| 4 | 30 | 61 |
| 2,2′ | 25.4 | 25 |
| 3,3′ | 25.4 | 21 |
| 4,4′ | 25.4 | 16 |
| 2 | 15 | 77 |
| 3 | 15 | 75 |
| 4 | 60 | 61 |

TABLE V

THE RELATIVE FLAME IONIZATION DETECTOR RESPONSE OF THE DECHLORINATION PRODUCTS OF 2',3,4- AND 2,4',5-TRICHLOROBIPHENYL

| *Structure of products* | *Starting material* | |
|---|---|---|
| | *2',3,4* | *2,4',5* |
| 0 | 30 | 22 |
| 2 | 46 | 2 |
| 3 | 10 | 29 |
| 4 | 22 | 45 |
| 2,5 | – | 8 |
| 2,3' | 27 | – |
| 2,4' | 48 | 16 |
| 3,4 | 14 | – |
| 3,4' | – | 75 |
| 2,4',5 | – | 100 |
| 2',3,4 | 100 | – |

dichlorinated rings are more readily attacked than the monosubstituted rings is not surprising given that the dichlorobiphenyls were found to be more reactive than the dichlorobiphenyls. This suggests that the electron density on the rings is a factor in determining the reactivity of the chlorines. Considering only the chlorines present on the dichlorinated rings it is likewise apparent that the *meta* position is more reactive than the *para* position in the overall dechlorination of 2',3,4-trichlorobiphenyl and similarly that the *ortho* position is more reactive than the *meta* position in the dechlorination of 2,4',5-trichlorobiphenyl. These orderings are the reverse of that seen for the mono- and dichlorobiphenyls. This demonstrates that the relative reactivity of the chlorines on a ring is dependent upon the other chlorines present. It should be kept in mind that the apparent reactivities of the positions may reflect the degree to which the substitution in that position influences adsorption in the catalyst and not only the absolute reactivity of that position.

The concentration of the PCB in the reaction mixture also affects the completeness of the reaction. In reacting 2,2',5,5'-tetrachlorobiphenyl at levels of 100, 50 and 25 $\mu$g the per cent concentration of unreacted starting material was reduced to trace levels and the per cent of biphenyl that could be formed was increased substantially as the concentration of PCB in the starting mixture was decreased (Table VI). This effect, believed to arise from competition for the active sites on the $Ni_2B$, can be used to control the extent of the reaction. The relative reactivity of the positions in the monochlorobiphenyls was not found to change when their relative concentrations were changed, but their total concentration was kept constant (Table IV).

The effect of varying the time of reaction from 0.5 min to 2 min shows that most of the reaction occurs in the early stages of the reaction (Table VI). This observation has also been made by Dennis and Cooper[16] in dechlorinating DDT by means of the $NaBH_4$–$Ni_2B$ system.

The dechlorination method described in this experimental section readily provides PCBs for use as GC retention time standards from PCBs containing four or less chlorines. The confidence in identifying and generating PCBs from the more

TABLE VI

RESULTS OF REACTING 2,2′,5,5′-TETRACHLOROBIPHENYL WITH SODIUM BOROHYDRIDE-NICKEL BORIDE AT VARYING CONCENTRATIONS AND TIMES

| *Starting concentration of 2,2′,5,5′ (μg)* | *Reaction time (min)* | *Remaining 2,2′,5,5′ (%)* | *Biphenyl formed (%)* |
|---|---|---|---|
| 100 | 1 | 7 | 7 |
| 50 | 1 | Trace | 18 |
| 25 | 1 | Trace | 25 |
| 100 | 0.5 | 7 | 7 |
| 100 | 2 | 4 | 7 |

highly chlorinated PCBs decreases as the chlorine content increases. The PCB identification procedures described in this paper may be used to help identify species not generated by the dechlorination procedure.

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