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DIFFERENTIATION AND CHARACTERIZATION OF ISOMERIC POLYCHLORINATED BIPHENYLS BY GAS–LIQUID CHROMATOGRAPHY COUPLED WITH ELECTRON IMPACT AND CHEMICAL IONIZATION MASS SPECTROMETRY*

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SUMMARY

This report describes the utilization of gas–liquid chromatography coupled with chemical ionization and electron impact mass spectrometry for the characterization and isomeric differentiation of polychlorinated biphenyls (PCB's) from numerous sources. By a comparison of model compounds with very complex mixtures, one is able to gain significant information about the *o,o'*-chlorine interaction of PCB's as monitored by mass spectrometry. The abundance of the specific $(M - Cl)^+$ ion assisted greatly in the isomeric differentiation of dichloro-, hexachloro-, and heptachlorobiphenyls. Furthermore, by the indepth characterization by mass spectrometry of the Ullman reaction products formed in the preparation of ^{14}C-labeled PCB's, one may conclude that a very complex mixture of dichloro- and trichlorobiphenyls is formed from a specific pure isomeric iodomonochlorobenzene during the Ullman reaction. As revealed by the use of gas–liquid chromatography coupled with electron impact mass spectrometry, care must be exercised in choosing the type of organic synthetic reactions for the preparations of polychlorinated biphenyls which will be used for biological testing in order to minimize isomeric contamination.

INTRODUCTION

Polychlorinated biphenyls (PCB's) along with 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDE) are reported to be the most abundant chlorinated aromatic pollutants in the global ecosystem[1]. PCB's have been found in extracts of sea-eagles, pike and salmon[1], in Canadian and British wildlife[2,3], in fish, mussels and birds from the River Rhine and The Netherlands' coast, and in wildlife of Sweden, England and the United States[3–7]. PCB's also have been reported in foods[8] and components of the food chain[8], in human adipose tissue[9], and in human milk[10].

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Contamination of rice bran oil with PCB's resulted in human fatalities and in fatal edema in 400,000 chicks in western Japan in 1968. Because of the high persistance of PCB's in the environment along with numerous clearly undesirable properties associated with human health, a more well defined knowledge of the chemistry and the potential health hazards of pure isomeric PCB's must be obtained. Interest in the environmental aspects of PCB's is growing rapidly. Recently, numerous review articles have appeared on this subject[11–18].

Many of the early investigations concerned with the biological exposure to and toxicology of PCB's were carried out with the complex commercial mixtures. Because of the degree of complexity of these commercial mixtures, a general lack of definition in respect of the biological activity of individual components of these mixtures exists.

The present investigations are part of a series to assist in obtaining clarity and definition in respect of the potential health hazards associated with specific pure isomeric PCB's. As will be presented, a more descriptive qualitative analytical tool consisting of gas–liquid chromatography (GLC) coupled with electron impact (EI) and chemical ionization (CI) mass spectrometry was investigated for the differentiation and characterization of individual PCB isomers. This powerful analytical tool also assisted in the clarification of the structure of numerous isomeric contaminants of organic reactions and in understanding the possible reaction mechanisms of syntheses used for the preparation of PCB's.

MATERIALS AND METHODS

The Aroclor 1260 was obtained from Monsanto (St. Louis, Mo., U.S.A.). All of the unlabeled pure isomeric tetrachloro-, hexachloro-, and heptachlorobiphenyls were synthesized as described earlier[19]. The ^{14}C-labeled 4,4'-dichlorobiphenyl and 2,2'-dichlorobiphenyl were prepared from the ^{14}C-labeled pure isomer of the appropriate chloroaniline, which was converted to the specific iodochlorobenzene, which then was reacted in the presence of copper to yield the ^{14}C-labeled biphenyls.

A more detailed procedure for the preparation of the ^{14}C-labeled 4,4'-dichlorobiphenyl is given below. 272.6 mg of unlabeled 4-chloroaniline hydrochloride (prepared from 4-chloroaniline (Aldrich, Milwaukee, Wisc., U.S.A.) followed by repetitive recrystallization) plus 55.6 mg of ^{14}C-labeled 4-chloroaniline, 4.00 mCi (Mallinckrodt, St. Louis, Mo., U.S.A.), was placed in a 100-ml round-bottom flask in an ice-bath and equipped with a magnetic stirrer and reflux condensor. Next 2.0 ml of concentrated hydrochloric acid was added to the reaction vessel. The mixture was stirred at 0–5° while a solution of sodium nitrite (1.0 g in 1.0 ml water) was added. After the addition was complete, the reaction mixture was stirred for an additional 30 min. Next 15 g of potassium iodide in 35 ml of water were added dropwise with vigorous stirring. The dark reaction mixture was then stirred at 45–55° in a water-bath for about 5 min, after which time a few drops of a saturated sodium bisulfite solution were added to clear up the dark color. Next, approximately 40 ml of diethyl ether were added to the reaction mixture followed by removal of the aqueous phase from the separatory funnel. The ether extract was dried over anhydrous sodium sulfate and decolorized with a small amount of Norit A decolorizing carbon. The ether was removed under vacuum to yield 361 mg (76% yield) of light yellow solid of 1-iodo-4-chloro[U-^{14}C]benzene.

The freshly prepared 1-iodo-4-chloro[U-^{14}C]benzene was placed in a thick-

walled glass reaction tube with 880 mg of activated copper dust[20]. The tube was evacuated and then sealed with a propane torch. The sealed tube was then heated at 225° for approximately 24 h. After cooling, the reaction tube and its contents were placed in a micro soxhlet extractor with ethanol as solvent in order to remove the desired components from the copper. The ethanol then was concentrated to yield the crude 4,4'-dichloro[U-^{14}C]biphenyl and numerous labeled contaminants. Column chromatography did not significantly enhance the purity of the desired component. The crude ^{14}C-labeled material was successfully recrystallized three times from methanol–water (60:40) to produce a very small quantity (22.53 mg) of purified 4,4'-dichloro[U-^{14}C]biphenyl with a specific activity of 3.92 mCi/mmole. By gas chromatography coupled with radioactivity monitoring of the effluent by a Model 894 gas proportional counter (Packard, Downers Grove, Ill., U.S.A.), the above purified ^{14}C-labeled 4,4'-dichlorobiphenyl contained only one radioactively labeled component with the same retention time as that of standard 4,4'-dichlorobiphenyl.

The above described procedure was also used to prepare the ^{14}C-labeled 2,2'-dichlorobiphenyl from ^{14}C-labeled 2-chloroaniline. As will be discussed later, care must be exercized with the utilization of the ^{14}C-labeled dichlorobiphenyls derived from this complex reaction for metabolic investigations unless one very vigorously analyzes the isomeric purity and radiopurity of these components before administration to biological systems.

The complexity and purity of all ^{14}C-labeled biphenyls were determined using a Tracor (Austin, Texas, U.S.A.) Model MT-220 gas chromatograph equipped with facilities for both simultaneous and separate determination of radioactivity by a gas proportional counter (Packard Model 894) and also total mass using a flame ionization detector. Once the distribution of the radioactivity was known, these samples were then analyzed by GLC coupled with mass spectrometry.

In the case of the ^{14}C-labeled 2,2'-dichlorobiphenyl reaction mixture, retention indices were determined on a 4-m stainless-steel column packed with 3% cyclohexanedimethanol succinate[21] on 100–120 mesh Gas-Chrom Q.

GLC–EI mass spectrometry

Unless otherwise stated EI mass spectra were obtained using a Finnigan (Sunnyvale, Calif., U.S.A.) Model 1015C Quadrupole mass spectrometer[21–24] interfaced with (1) a Varian Aerograph (Palo Alto, Calif., U.S.A.) Model 1400 gas chromatograph or more recently with (2) a Finnigan Model 9500 gas chromatograph and all-glass transfer interface line with single-stage glass jet separator. The manifold temperature was maintained at 175°. Routinely spectra were collected at 70 eV, 20 eV and occasionally at 10 eV with 3000 V applied from the high-voltage power supply. With either of the above interfaced gas chromatographs, a 152 × 0.2 cm I.D. glass column packed with 3% OV-1 on Gas-Chrom Q (80–100 mesh) with a measured helium flow-rate of 35–40 ml/min was used with an overall pressure of 5×10^{-5} to 10^{-6} Torr. The injector temperature was maintained at 250°.

GLC–CI mass spectrometry

All CI mass spectra were obtained with a Finnigan Model 1015C Quadrupole with (1) a Varian Aerograph Model 1400 gas chromatograph or more recently with (2) a Finnigan Model 9500 gas chromatograph. The manifold temperature was main-

tained at 175° with an interface temperature of 225°. The ionizer heater was kept off in order to minimize ion fragmentation due to thermal effects. Under the above conditions, the ionizer temperature because of manifold heat transfer is only 50–75° maximally. For methane or isobutane, the ion source pressure is maintained at 600–1000 μ with an overall pressure of about 10^{-5} Torr. The ion source conditions were as reported earlier[22–24].

After injection of the desired sample in the specific solvent, the ionizer is maintained off a specified duration of time. The GLC column conditions were as indicated for EI.

Data acquisition

This dual Finnigan Model 1015 EI and CI mass spectrometer system operated from a common electronic console is controlled by a System 150 data collection system (System Industries, Sunnyvale, Calif., U.S.A.) composed of a PDP-8E, magnetic tape drive, disk, plotter, teletype, and interfacing hard- and software. This laboratory is in the unique position of having both the EI and the CI system from the same company, so that they can be interchangeably interfaced to one computer system for collecting and processing of data. This enables one to more confidently make comparison statements regarding the advantages and disadvantages of one system over the other with a given series of compounds and other variables being the same.

RESULTS AND DISCUSSION

Commercial PCB's are produced by chlorination of the biphenyls with anhydrous chlorine using either ferric chloride or iron as catalyst. In this process of replacing hydrogen atoms with those of chlorine, theoretically 210 compounds could be formed[18]. The commercial uses of PCB's include coolant insulator fluids in transformers and capacitors, plasticizers in wire and cable coatings, impregnation of cotton and asbestos for electrical wiring, insulation, ballasts for fluorescent fixtures, high-pressure hydraulic fluid, heat transfer agents, machine cutting oils, lubricants and gasket sealants, and incorporation into formulations in epoxy paints, resins, chlorinated rubber, printer's ink, waxes, adhesives, textile dyes and in carbonless reproducing paper.

The toxicology of PCB's presently is very poorly defined. The most important effects caused by PCB's seem to be long-range sublethal in nature. In general, the pathological changes in mammals include alterations in the liver, accumulation of fluid in the pericardial sac, kidney damage and possible reduction in the spleen[18]. Most of the results concerning toxicity and effects on biological systems have been obtained with the very complex commercial mixtures[16–18]. A clear understanding of the biological activity of individual isomeric components of the commercial PCB's is not available at this time. As exemplified by numerous biological samples, some PCB isomers of the commercial PCB's seem to be concentrated in biological systems while still other isomers are non-existent. Only after thorough investigations concerning the metabolsim and toxicity of pure isomeric polychlorinated biphenyls have been completed, will one be able to indicate specifically the potential health hazards associated with specific isomeric PCB's.

Fig. 1 illustrates the complexity of one of the commercial Aroclors which has

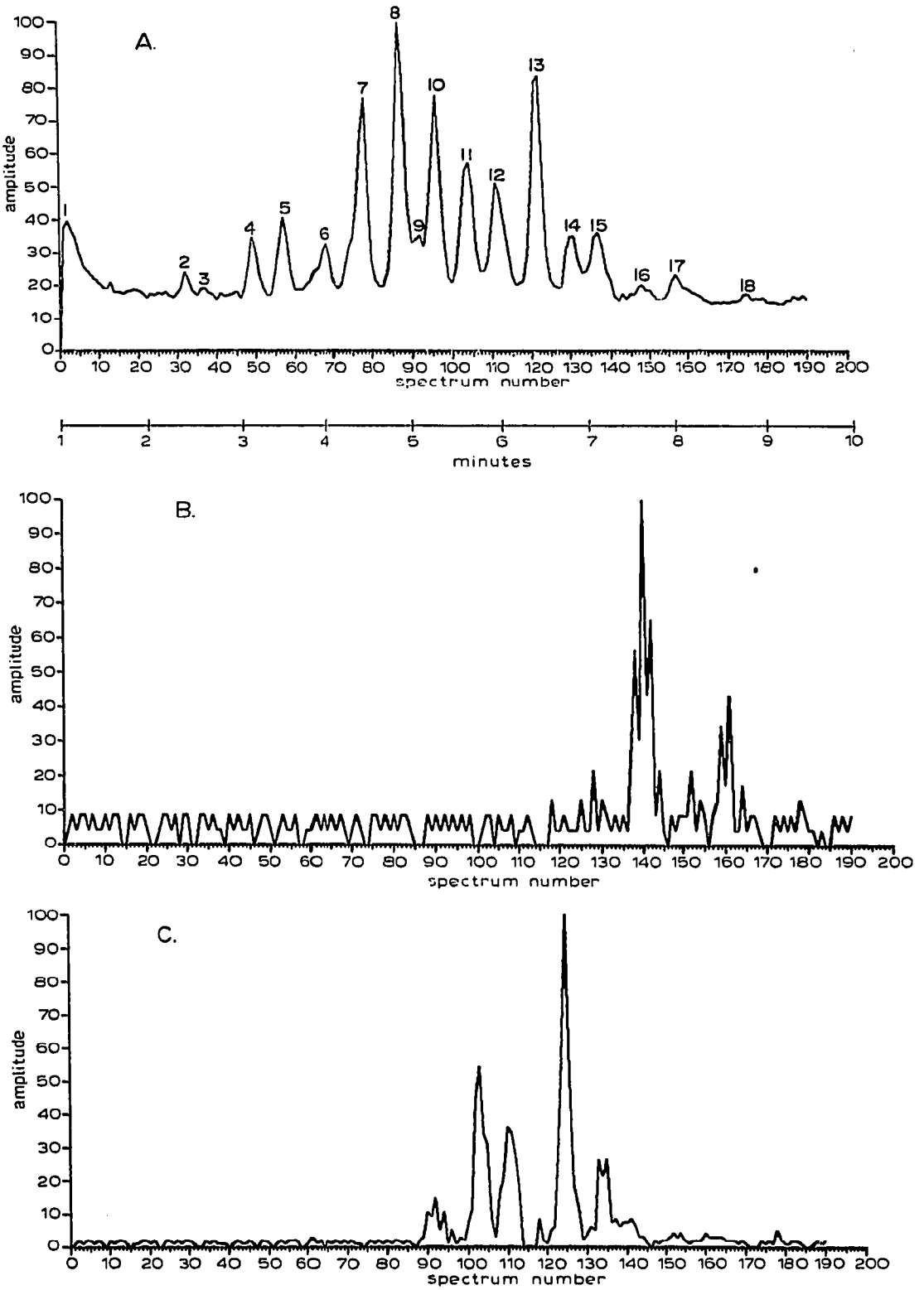

Fig. 1.

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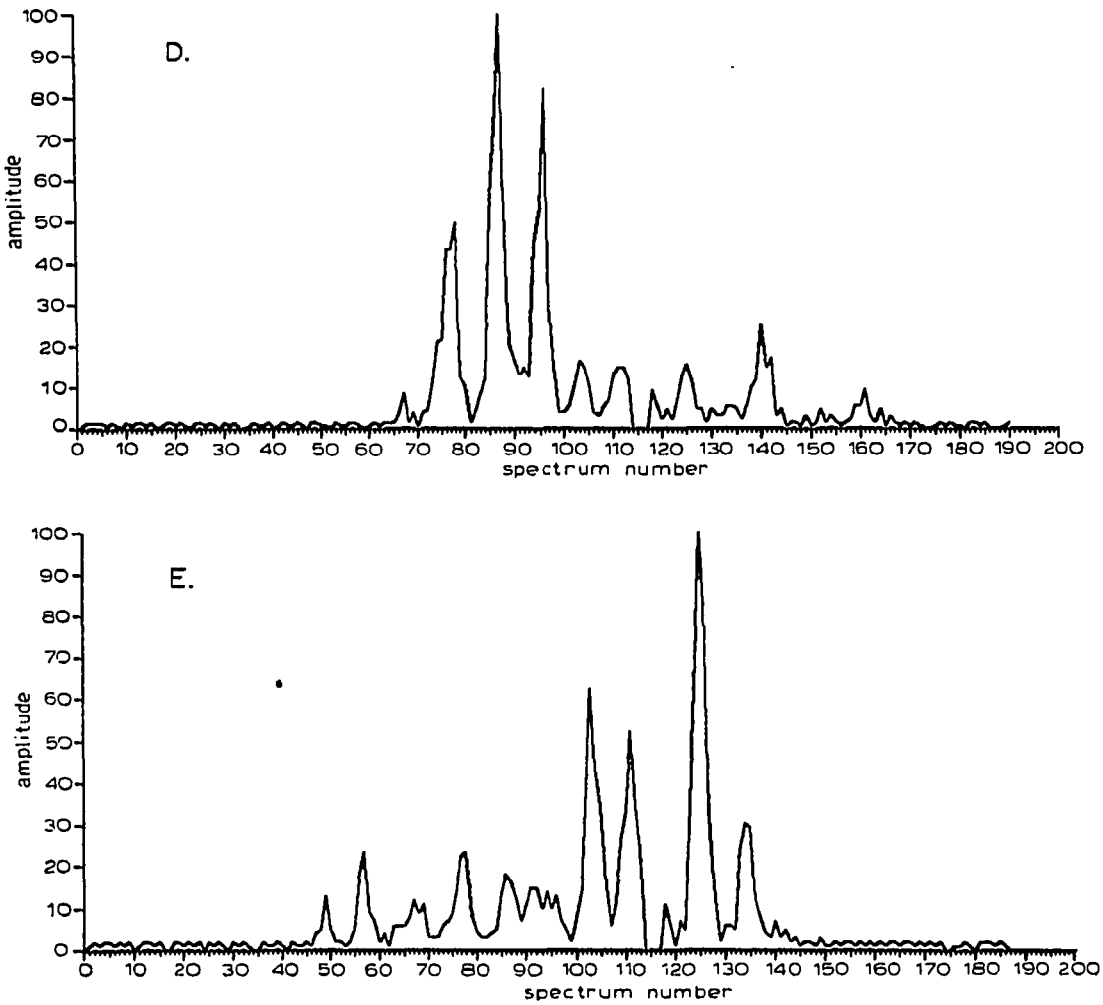


Fig. 1. (A) Reconstructed gas chromatogram of Aroclor 1260. Approximately 10 μg of Aroclor 1260 were injected on a 3% OV-1 column at 200°. The ionizer was initiated 1 min after injection, at which time the column temperature was programmed from 200–250° at a rate of 10°/min. 70-eV EI mass spectra were collected continuously from 1 to 10 min after injection. (B) Limited mass search *m/e* 426–428. (C) Limited mass search *m/e* 392–394. (D) Limited mass search *m/e* 358–360. (E) Limited mass search *m/e* 322–324.

been used for numerous toxicity and biological investigations[16–18]. Many isomeric components similar to those in the Aroclor 1260 have been identified in human adipose tissue[9], human milk[10], in sea-eagles, pike and salmon[1], in sea-water and phytoplankton[25], in numerous land and sea animals, and in biological components of the food chain[16–18]. As indicated by Fig. 1, Aroclor 1260 is composed mainly of isomeric hexachlorobiphenyls and heptachlorobiphenyls with lower concentrations of the higher and lower chlorinated biphenyls.

As will be illustrated later for pure isomeric PCB's, *o,o'*-chlorinated biphenyls, which have an asymmetric distribution of chlorine atoms on the aromatic ring,

characteristically form very abundant $(M - Cl)^+$ ions during analysis by mass spectrometry. This interaction of chlorine atoms does not seem to be caused by interaction of *o*-chlorines from the same aromatic ring but seems to be caused by the *ortho–ortho* interaction between chlorine atoms of adjacent aromatic rings. Furthermore, this tendency for expulsion of a chlorine atom caused by this *ortho–ortho* interaction seems to be present only in instances of asymmetric chlorinated biphenyls. We have observed this effect of *o*-chlorine interaction by both EI and CI mass spectrometry.

Of the major hexachloro- and heptachlorobiphenyls present in Aroclor 1260, *o,o'*-chlorine substitution with the above characteristics does seem to exist. A comparison of mass spectra of two separable hexachlorobiphenyls of Aroclor 1260 is shown in Figs. 2A and B. With reference to Fig. 1A, peak 7 (spectrum 78) seems to contain an *o,o'*-substituted biphenyl with a very abundant $(M - Cl)^+$ ion (47%) whereas peaks 8 or 10 do not (Fig. 2B). In respect of the heptachlorobiphenyls of Aroclor 1260, peak 12 has characteristics similar to peak 7 of this mixture with a very abundant $(M - Cl)^+$ ion. Very similar characteristic mass spectra which have very abundant $(M - Cl)^+$ ions have been reported for various hexachlorobiphenyls found in human adipose tissue[9], in human milk, in the sea-eagle, and in other land and sea animals[16–19]

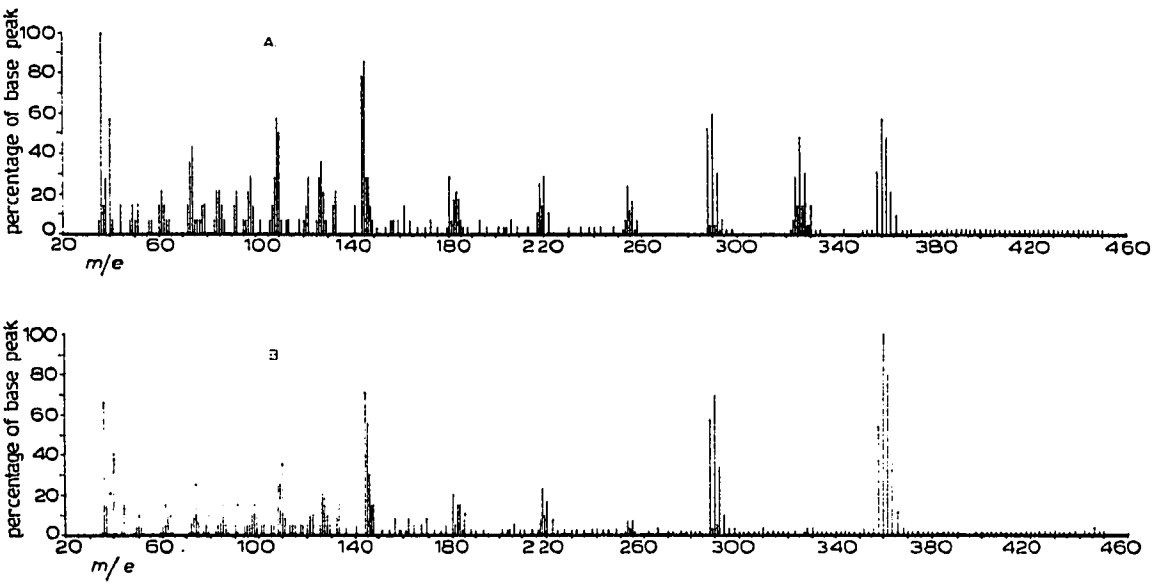
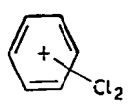
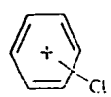


Fig. 2. 70-eV EI mass spectrum of a hexachlorobiphenyl in Aroclor 1260. (A) Spectrum 78–74 of Fig. 1A; (B) spectrum 96–91 of Fig. 1A.

Because of the complexity of the Aroclor mixture, the above mass spectral characteristics were investigated further with numerous pure isomeric PCB's. Fig. 3 illustrates the chemical structures of some of the isomeric PCB's which were investigated by GLC coupled with EI and CI mass spectrometry. Using a specific directed synthesis for asymmetrical PCB's, further insight was gained into the interaction phenomenon of *o,o'*-chlorine atoms of various hexachlorobiphenyls[19], Tables I and II illustrate the characteristics of some of these pure isomers as monitored by mass

TABLE I

CHARACTERISTIC ABUNDANCE OF VARIOUS IONS DERIVED FROM ASYMMETRICAL PCB ISOMERS ANALYZED BY GLC COUPLED WITH CI AND EI MASS SPECTROMETRY

Isomer	$(M-Cl)^+$									$(M-Cl_2)^+$			$(M)^+$
	m/e	*EI* (%)*	*CI** (%)*	*m/e*	*EI (%)*	*CI (%)*	*m/e*	*EI (%)*	*CI (%)*	*m/e*	*EI (%)*	*CI (%)*	*m/e*
2,3,4,5,6	290	2.8	0	145	16.5	0	109	13.8	0	254	58.7	1.2	324
2,3,4,5,6-2′	325	50.8	2.0	145	73.7	0	109	34.4	0	290	100	2.1	358
2,3,4,5,6-3′	325	2.8	0.6	145	50.7	0	109	21.1	0	290	77.5	1.2	358
2,3,4,5,6-4′	325	0.8	0	145	50.6	0	109	23.4	0	290	81.8	1.2	358
2,3,4,5,6-3′,4′	358	1.2	0	145	11.1	0	109	25.9	0	322	40.7	0	392

* 70-eV EI mass spectra.

** Methane CI mass spectra.

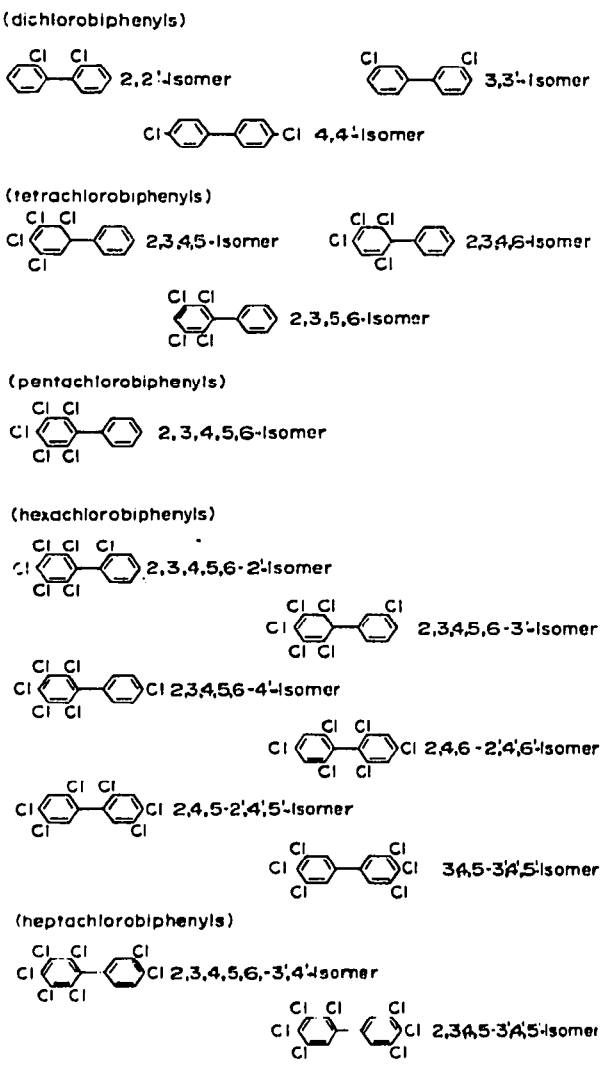


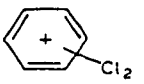
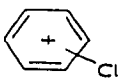
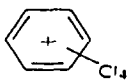
Fig. 3. Chemical structures of isomeric polychlorinated biphenyls investigated by GLC coupled with mass spectrometry.

spectrometry. As can be seen from Table I, there is a very abundant $(M - Cl)^+$ ion for the 2,2'-chlorine-substituted hexachlorobiphenyls. On the contrary, other asymmetrical chlorine-substituted isomers which do not have the *o,o'* substitution also do not exhibit the abundant $(M - Cl)^+$ ion.

The explanation for the great abundance of the $(M - Cl)^+$ ion specifically for the hexachlorobiphenyls is that it is not caused simply by *ortho–ortho* interaction of chlorine atoms. As can be seen for the symmetrical hexachlorobiphenyls, components which have the 2,2'-chlorine substitution or even the 2,2'- and 6,6'-chlorine substitution do not exhibit this characteristic in their mass spectra (Table II). In addition, one may note that with the symmetrical hexachlorobiphenyls there is a very abundant (up

TABLE II

CHARACTERISTIC ABUNDANCE OF VARIOUS IONS DERIVED FROM SYMMETRICAL HEXACHLOROBIPHENYLS ANALYZED BY GLC COUPLED WITH EI MASS SPECTROMETRY

Isomer	$(M - Cl)^+$						$(M - Cl_2)^+$		$(M)^+$		
	m/e	EI* (%)	m/e	EI (%)	m/e	EI (%)	m/e	EI (%)	m/e	m/e	EI (%)
2,3,6-2',3',6'	325	6.5	145	97.8	109	65.2	290	76.1	358	218	29.3
2,4,5-2',4',5'	325	11.1	145	44.4	109	63.0	290	87.7	358	218	38.9
2,4,6-2',4',6'	325	2.0	145	94.4	109	69.2	290	72.0	358	218	34.6
3,4,5-3',4',5'	325	0	145	50.0	109	75.0	290	66.7	358	218	40.6

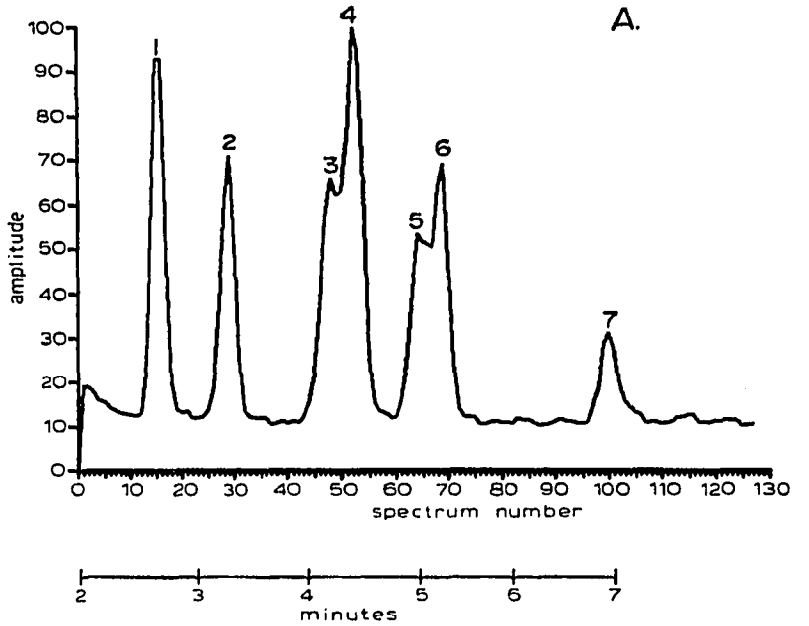
* EI mass spectra.

to 40%) ion *m/e* 218, possibly being derived from chlorine transfer from the adjacent ring to yield $(Cl_4C_6H_3)^+$.

Analyses of pure tetrachlorobiphenyl isomers with 2,3,4,5-, 2,3,4,6-, or 2,3,5,6-chlorine substitution indicate that there are additional factors that affect the abundance of the $(M - Cl)^+$ ion other than *ortho* substitution, as is also the case with the symmetric hexachlorobiphenyl isomers. In these tetrachlorobiphenyl isomers there is no significant abundance (<5%) of the $(M - Cl)^+$ ion in their mass spectra. It seems that the presence of the $(M - Cl)^+$ ion for numerous PCB's is a characteristic associated not only with *o,o'*-chlorine substitution but also with the number of chlorine atoms on the aromatic ring. This postulated requirement for the presence of numerous chlorine atoms, possibly more than three, on the aromatic ring may be related to the ability of the chemical species to distribute the positive charge throughout the aromatic ring in turn without expulsion of a second chlorine atom. A more detailed discussion will be given in a separate report on the effects of *ortho* substitution on the chemistry and reactivity of PCB's.

As can be seen from both the complex Aroclor 1260 and the pure asymmetric hexachlorobiphenyl, the characteristic $(M - Cl)^+$ ion is very useful from the point of view of analytical differentiation. Fig. 4 illustrates further with the component mixture of the earlier described asymmetrical and symmetrical hexachlorobiphenyls how this characteristic may be used to further differentiate components which may not be completely separable by GLC. The composite peak, spectra 40–57 of Fig. 4A, represents the 2,3,4,5,6-2'-isomer (peak 3) and the 2,4,5-2',4',5'-isomer (peak 4). By using the limited mass search for the ion fragment *m/e* 324–326, one can very easily distinguish the *o,o'* asymmetrical isomer from the symmetrical isomer (Figs. 4B and C). As indicated above, once it has been found that a particular chromatographic peak contains only hexachlorobiphenyls or heptachlorobiphenyls, the heterogeneity of isomeric components under this peak may be determined by computer assistance with numerous searches for specific mass ions.

A summary of the relative retention times of components of Aroclor 1260 (Fig. 1A), and various purified isomeric PCB's is given in Table III. By use of a combination of GLC retention properties and information acquired from mass spectra, one can identify and characterize isomeric PCB's from numerous sources. With this particular



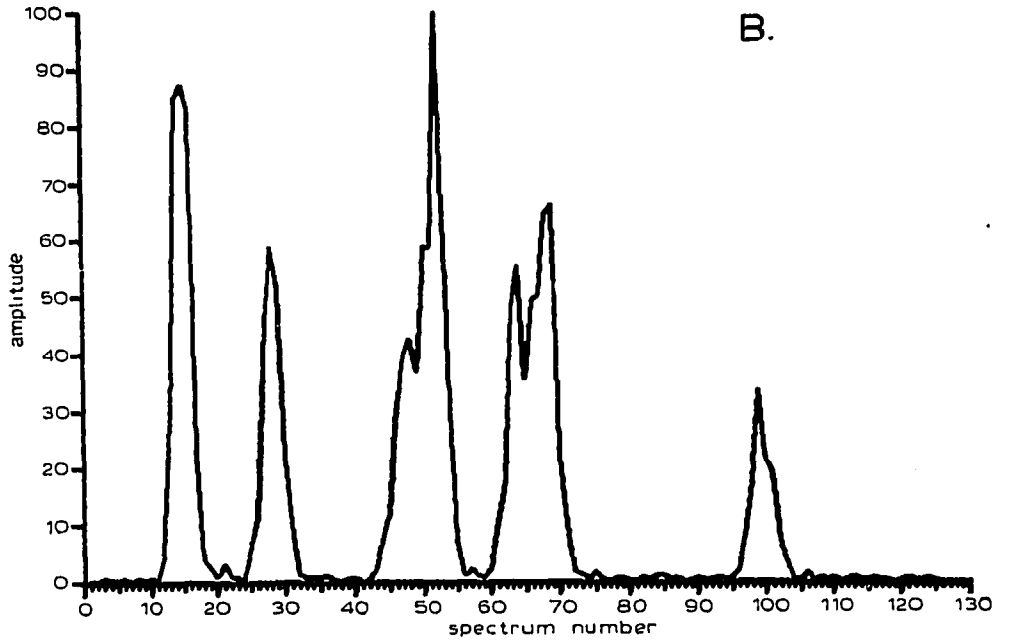


Fig. 4.

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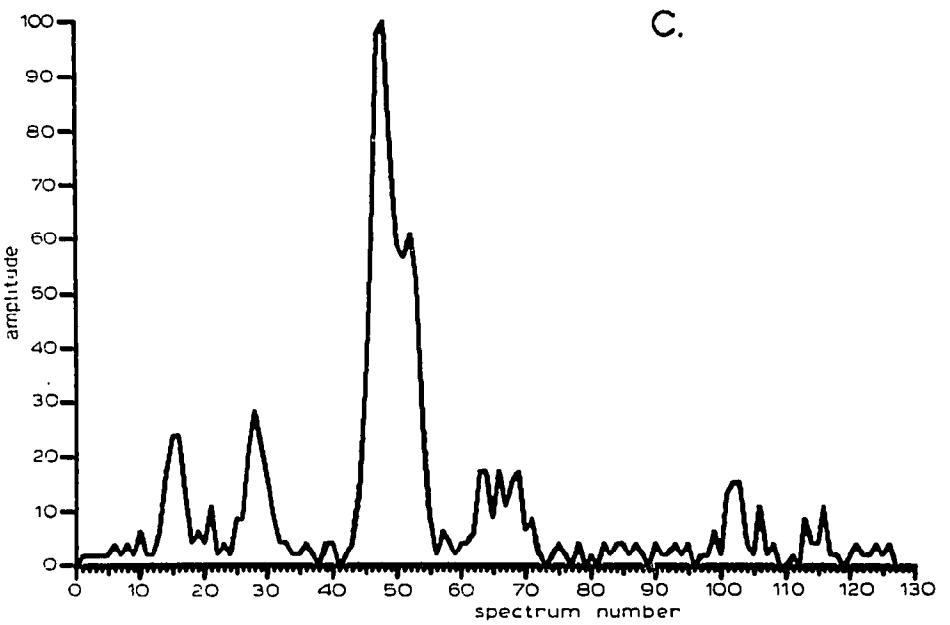


Fig. 4. (A) Reconstructed gas chromatogram of a standard mixture of hexachlorobiphenyls analyzed by GLC coupled with EI mass spectrometry. Analytical conditions, as in Fig. 1. Hexachlorobiphenyl isomers: (peak 1) 2,4,6-2′,4′,6′-isomer; (peak 2) 2,3,6-2′,3′,6′-isomer; (peak 3) 2,3,4,5,6-2′-isomer; (peak 4) 2,4,5-2′,4′,5′-isomer; (peak 5) 2,3,4,5,6-3′-isomer; (peak 6) 2,3,4,5,6-4′-isomer; (peak 7) 3,4,5-3′,4′,5′-isomer. (B) Limited mass search *m/e* 358–362. (C) Limited mass search *m/e* 324–326.

class of environmental agents, the EI mass spectra are much more informative than the CI spectra.

From the above description of the analyses of purified isomeric PCB's, one is able to obtain a large amount of useful information concerning the structure, possible chemical reactivity and desirable analytical techniques needed for a more thorough understanding of these widely distributed environmental agents.

The remaining portion of this discussion will be limited to the use of GLC and EI mass spectrometry for the determination of the presence of contaminants in synthetic PCB's prepared by various chemical procedures.

One of the common reactions used for the general synthesis of polychlorinated biphenyls is the chlorination of benzidine[26] with ultimate replacement of the nitrogen functions with chlorine. A short report[27] recently described some of the major by-products of this chlorination reaction. Fig. 5 illustrates the products of this reaction as analyzed by mass spectrometry. The major component (peak 1) is the 3,4,5-3′,4′,5′-hexachlorobiphenyl. A major contaminant of the hexachlorobiphenyl is the 2,3,4,5-3′,4′,5′-heptachlorobiphenyl[27], as shown in Fig. 5C. This heptachlorobiphenyl has a retention time relative to 3,4,5-3′,4′,5′-hexachlorobiphenyl of 1.38. The heptachlorobiphenyl, as predicted, does not exhibit a significant $(M - Cl)^+$ ion as monitored by EI mass spectrometry. The heptachlorobiphenyl does possess one of the postulated requirements for the formation of the abundant $(M - Cl)^+$ ion, *i.e.* the presence of more than three chlorine atoms on an aromatic ring. On the contrary, this heptachlorobiphenyl isomer does not possess the 2,2′-chlorine substitution.

TABLE III

CHROMATOGRAPHIC PROPERTIES OF POLYCHLORINATED BIPHENYLS AS DETERMINED BY GLC COUPLED WITH EI MASS SPECTROMETRY

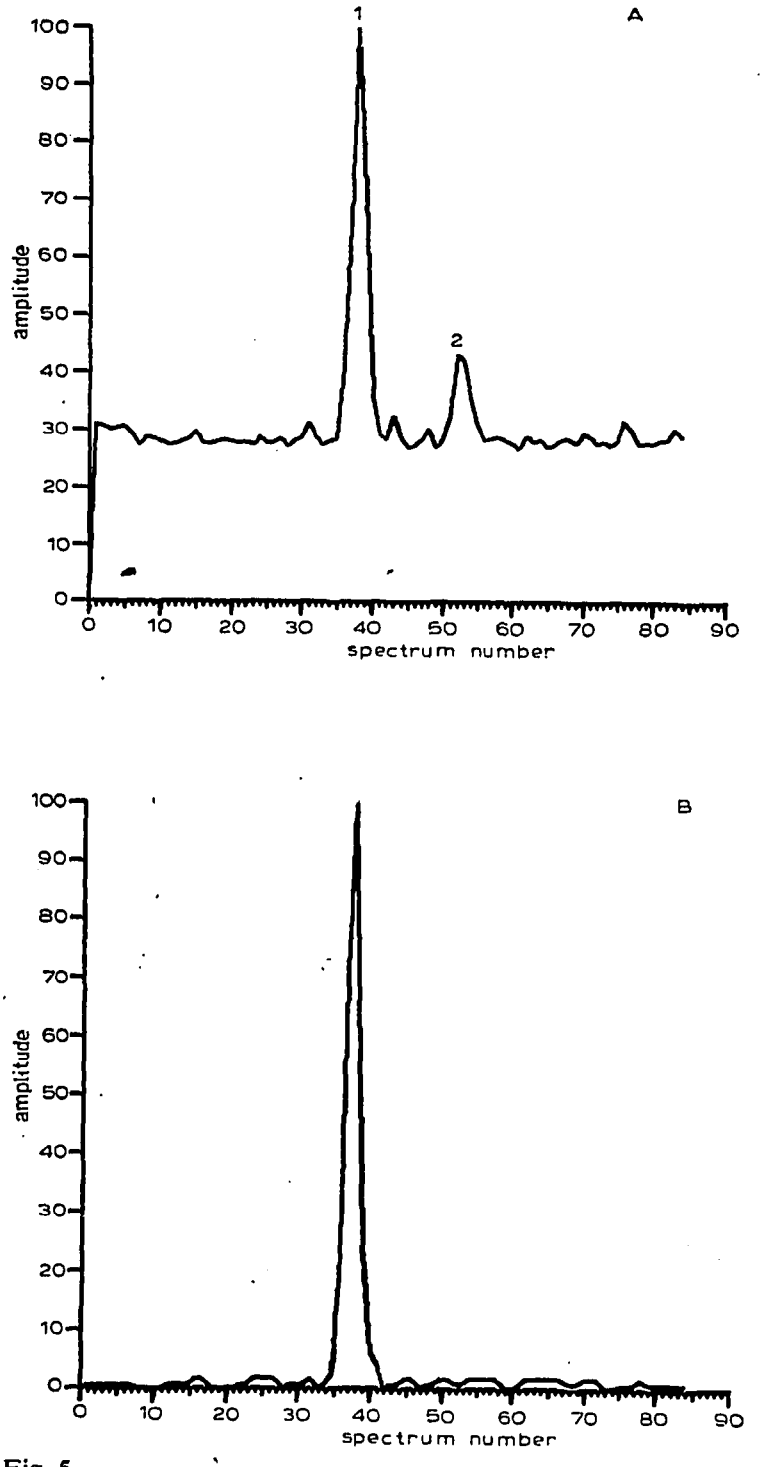
Isomer	*Relative retention time**	*Comments*
2,4,6-2′,4′,6′	1.00	Pure isomeric synthetic standard compounds
2,3,6-2′,3′,6′	1.39	
2,3,4,5,6-2′	1.91	
2,4,5-2′,4′,5′	2.02	
2,3,4,5,6-3′	2.37	
2,3,4,5,6-4′	2.50	
3,4,5-3′,4′,5′	3.35	
2,3,4,5,6-3′,4′	3.37	
Aroclor 1260		
Peak** 1	0.07	
2	0.87	
3	1.00	
4	1.35	Pentachlorobiphenyls
5	1.57	
6	1.87	
7	2.15	Hexachlorobiphenyls
8	2.39	
9	2.52	Heptachlorobiphenyls
10	2.65	Hexachlorobiphenyls
11	2.87	Heptachlorobiphenyls
12	3.07	
13	3.37	
14	3.59	
15	3.76	Octachlorobiphenyls
16	4.09	
17	4.30	
18	4.80	

* Retention time expressed relative to the 2,4,6-2′,4′,6′-hexachlorobiphenyl isomer.
** Analytical conditions as indicated for Fig. 1.

Most procedures used in the purification of synthetic PCB's do not discreetly remove the normal isomeric by-products of the reaction. One of the major reasons for development of more controlled and directed syntheses[19] of PCB's is to minimize the presence of isomeric contaminants in order to ensure a more valid biological investigation with a specific pure isomeric PCB.

In addition to preparing unlabeled pure isomeric PCB's to be used: (a) in the development of analytical methodology, (b) for investigation of the chemical reactivity, and (c) for toxicity investigation, we have been involved in developing and adapting synthetic procedures for the synthesis of ^{14}C-labeled monochloro- and dichlorobiphenyls. The present report will describe information that was obtained during the investigation of the feasibility of various types of reactions for the production of pure ^{14}C-labeled PCB's.

One of the more commonly used organic synthetic reactions for the synthesis of PCB's is the Ullman reaction[28–31]. The specific iodochlorobenzene is reacted at high temperature in a sealed tube with activated copper dust to yield the chlorinated


A
1
2
amplitude
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B
amplitude
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Fig. 5.

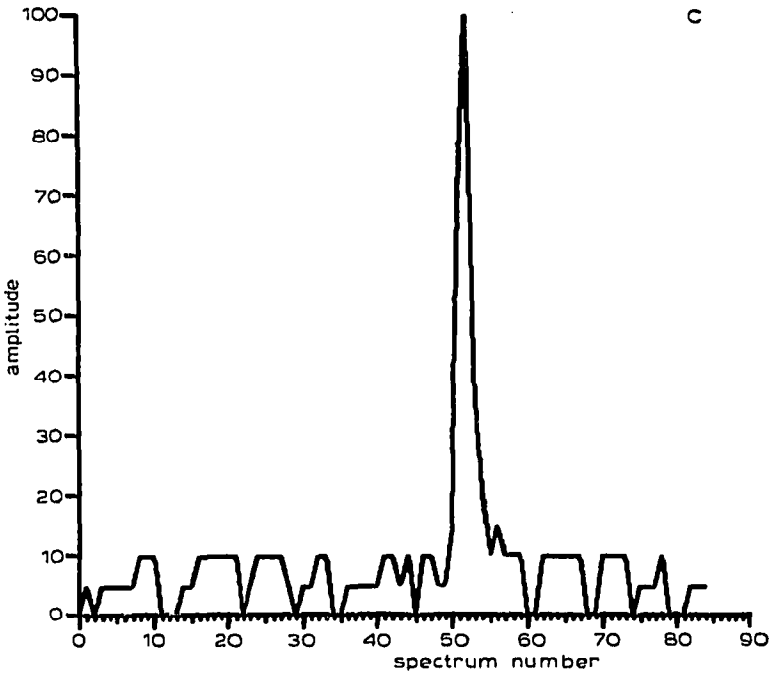


Fig. 5. (A) Reconstructed gas chromatogram of 3,4,5-3′,4′,5′-hexachlorobiphenyl prepared by chlorination of benzidine[26]. The crude reaction mixture was analyzed by GC–MS (70-eV EI) on 3% OV-1 as described for Fig. 1. The ionizer was initiated 4 min after injection and spectra were collected. Peak 1 is the 3,4,5-3′,4′,5′-isomer. Peak 2 is the 2,3,4,5-3′,4′,5′-isomer[27]. (B) Limited mass search *m/e* 360–362. (C) Limited mass search *m/e* 394–396.

biphenyl. Normal purification usually is carried out by recrystallization until one has obtained the desired component in the required purity.

As described in the methodology of this report, the appropriate isomeric ^{14}C-labeled monochloroaniline was converted to the appropriate pure isomeric iodochlorobenzene. The ^{14}C-labeled 1-iodo-4-chlorobenzene was then reacted with copper dust for the specified period of time. Prior to carrying out a specific labeling reaction, the synthetic reaction conditions were idealized with unlabeled components followed by recrystallization to generate the final pure specific dichlorobiphenyl isomer in a low yield (10–40%). Routine analysis of final purified unlabeled dichlorobiphenyl isomer would yield one component by GLC with the desired mass spectrum and the appropriate retention time.

Upon synthesis of the ^{14}C-labeled dichlorobiphenyl, many very undesirable properties became very apparent immediately. With the increased sensitivity of radiolabeling, reaction components which were below routine detection limits using the flame ionization detector became very apparent and troublesome using the gas flow proportional detector. Fig. 6 represents the chromatogram of the crude reaction mixture of ^{14}C-labeled 4,4′-dichlorobiphenyl prior to analysis by EI mass spectrometry.

As indicated by Fig. 6A, all of the components contained radiolabel. Traces of the unreacted ^{14}C-labeled 1-iodo-4-chlorobenzene are present in the solvent front. The remaining radioactive components were characterized by mass spectrometry.

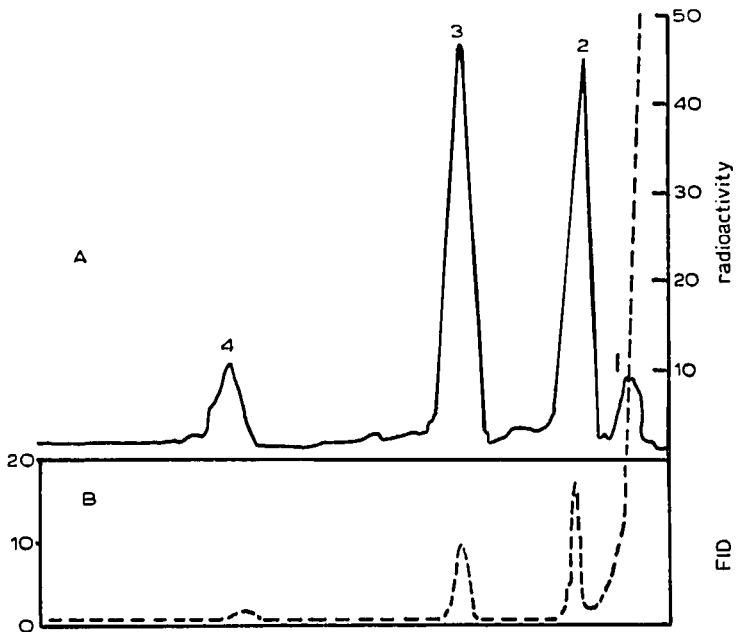


Fig. 6. Gas chromatogram of the crude Ullman reaction mixture formed during preparation of ^{14}C-labeled 4,4′-dichlorobiphenyl. A 184 × 0.6 cm glass column packed with 11% OV-17–QF-1 mixed phase on 80–100 mesh Gas-Chrom Q was used. Helium flow-rate, 50 ml/min; injector temperature, 250°; flame ionization detector temperature, 250°; inlet temperature to proportional detector, 250°; furnace temperature, 700°; column temperature, 160° isothermally. (A) Distribution of radioactivity; range, 10 K; time constant, 2; discriminator mode, A-α; background offset, 0. (B) Flame ionization detector response for the ^{14}C-labeled reaction mixture.

Fig. 7A represents the reconstructed gas chromatogram from the mass spectrometer for the crude ^{14}C-labeled 4,4′-dichlorobiphenyl mixture as obtained by the Ullman reaction. The ^{14}C-labeled 4,4′-dichlorobiphenyl under these analytical conditions eluted in 5½–6 min (spectra 114–129). The major contaminant of this reaction is the monochlorobiphenyl (spectra 37–48) as indicated by the limited mass search *m/e* 188 (Fig. 7D). This monochlorobiphenyl has a retention time relative to the ^{14}C-labeled 4,4′-dichlorobiphenyl of 0.35. Experiments that will be discussed later indicate that by the use of freshly prepared copper dust, the reduction product, namely the monochlorobiphenyl, can be minimized. Two very unexpected contaminants of the Ullman reactions are an additional isomeric dichlorobiphenyl (spectra 84–94) and a trichlorobiphenyl (spectra 182–190). Both of the isomeric contaminants are very visible, as indicated by Figs. 7A–D. The retention time of the isomeric dichlorobiphenyl contaminants is 0.74 and that of the ^{14}C-labeled trichlorobiphenyl is 1.52 relative to the 4,4′-dichlorobiphenyl. The source and problems associated with the presence of these contaminants produced by the Ullman reaction will be considered further in the discussion of the ^{14}C-labeled 2,2′-dichlorobiphenyl.

By careful control of the time of initiation of the ionizer, the major component of the ^{14}C-labeled 4,4′-dichlorobiphenyl mixture (Fig. 7C) may be eliminated from the chromatogram. By using this technique, one may study components in the mixture which are present in the low nanogram or even in picogram quantities. Fig. 8 illus-

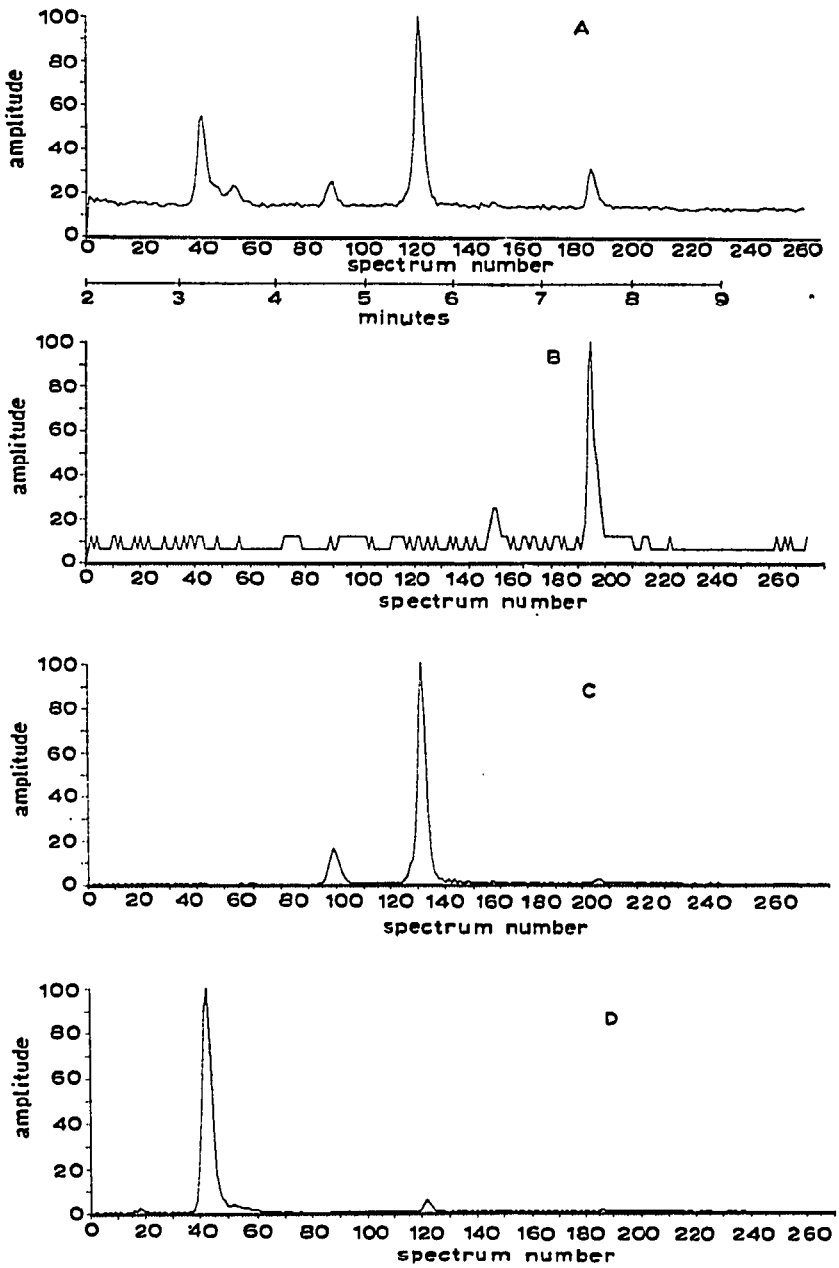
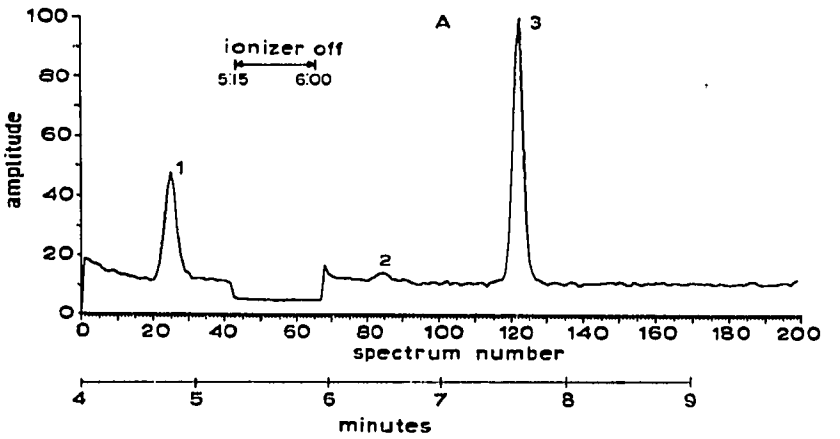
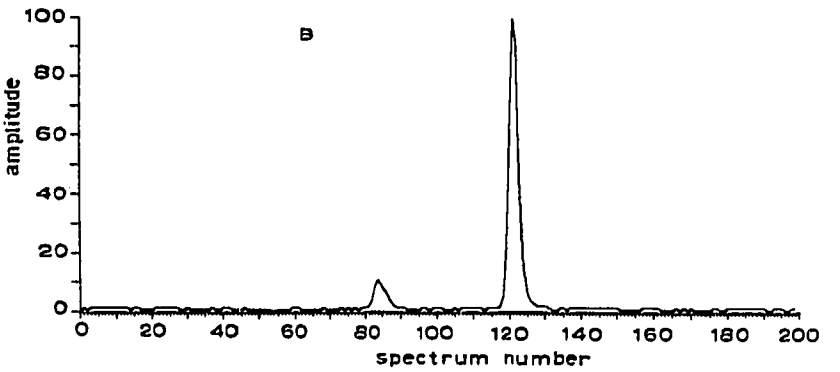


Fig. 7. (A) Reconstructed gas chromatogram of the Ullman reaction mixture generated during the preparation of ^{14}C-labeled 4,4'-dichlorobiphenyl. The mixture was analyzed by GLC–EI mass spectrometry (70 eV) on a 3% OV-17 glass column (152 × 0.2 cm I.D.) with a helium flow-rate of 35–40 ml/min. The sample was injected on the column at 140°. After 1 min the column temperature was programmed from 140–220° at 10°/min. The ionizer was initiated 1 min after injection. Spectra were collected for the specified period of time. (B) Limited mass search *m/e* 256–258. (C) Limited mass search *m/e* 222. (D) Limited mass search *m/e* 188.





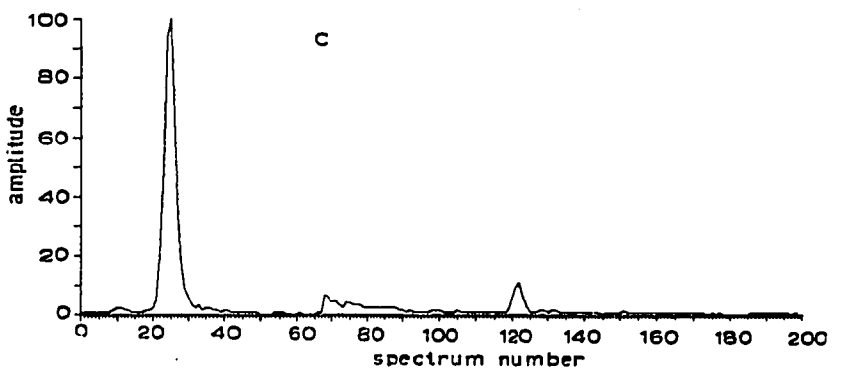


Fig. 8. (A) Reconstructed gas chromatogram of the contaminants of the ^{14}C-labeled 4,4'-dichlorobiphenyl synthesis analyzed with controlled initiation of the ionizer. The analytical conditions were as described in Fig. 7A except that 4 min after injection of the sample, the ionizer was initiated. Spectra were collected from 4–10 min. At 5 min 15 sec after injection the ionizer was turned off and kept off until 6 min, at which time it was re-initiated. (B) Computer limited mass search *m/e* 256–258. (C) Computer limited mass search *m/e* 222.

trates the use of controlled initiation of the ionizer for detection of low trace contaminants coupled with the use of the computer limited mass search. In Fig. 8A the ionizer was initiated 4 min after injection of the sample. Spectra were collected from 4 min up to 5 min 15 sec, at which time the ionizer was turned off. During the period from 5 min 15 sec to 6 min, the computer continued to scan with the ionizer off. At 6 min the ionizer was re-initiated and spectra were collected for the remaining period. The contaminant which was not detected earlier seems to be an isomeric trichlorobiphenyl (spectra 81–88, Fig. 8B), with a retention time relative to 4,4′-dichlorobiphenyl of 1.06.

Representative EI mass spectra of the major isomeric contaminants of ^{14}C-labeled 4,4′-dichlorobiphenyl which were prepared by the Ullman reaction are given in Figs. 9A, B and C.

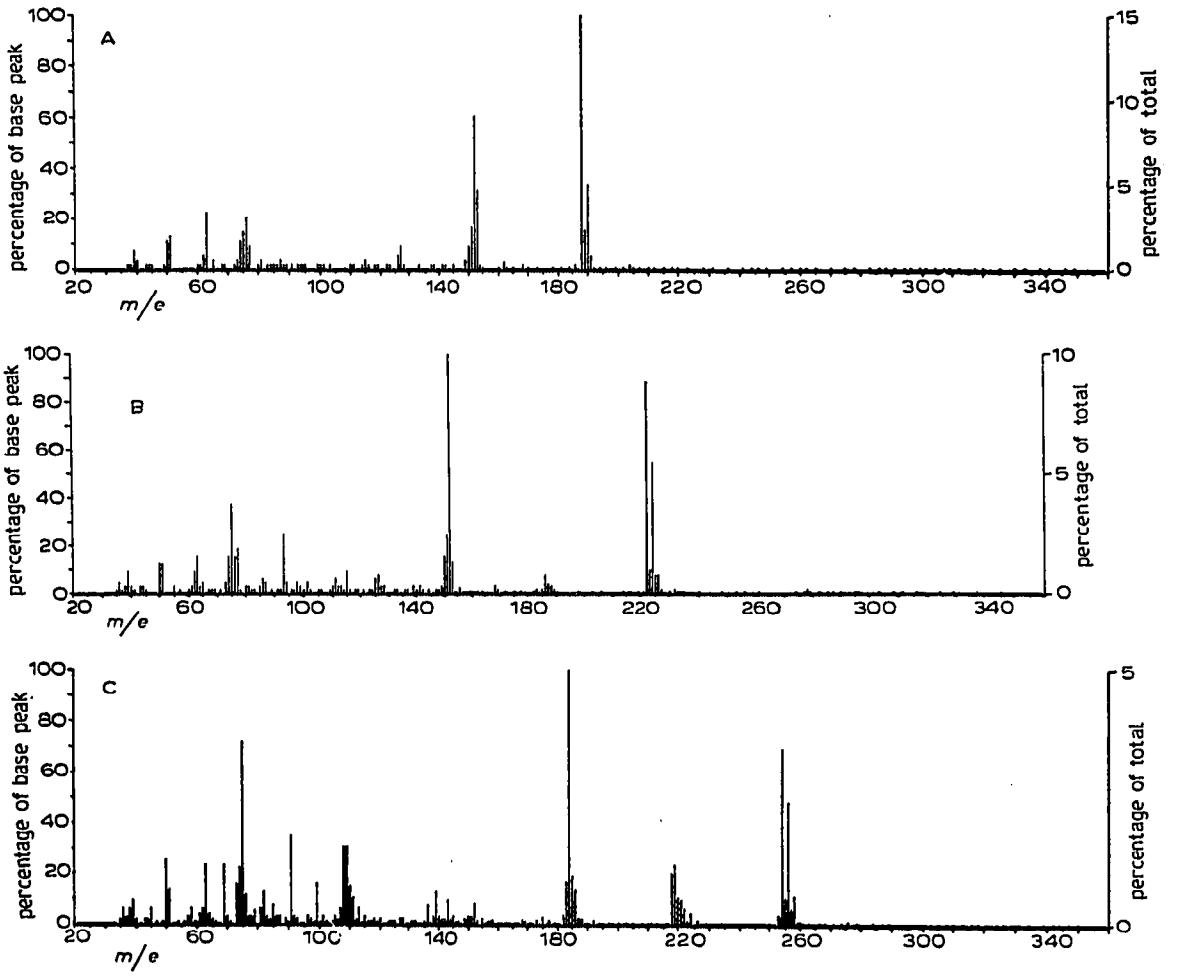


Fig. 9. 70-eV EI mass spectra of the major contaminants of the Ullman reaction in the synthesis of ^{14}C-labeled 4,4′-dichlorobiphenyl when the copper dust was not freshly prepared. (A) Reduction product, the monochlorobiphenyl (spectrum 42–36; Fig. 7A). (B) Isomeric dichlorobiphenyl contaminant (spectrum 25–19; Fig. 8A). (C) Isomeric trichlorobiphenyl contaminant (spectrum 122–116; Fig. 8A).

Even though this mixture (Fig. 7A) is highly contaminated with isomeric monochloro-, dichloro-, and trichlorobiphenyls, one can obtain by repetitive recrystallization a ^{14}C-labeled dichlorobiphenyl in pure form which has only one radioactive component and which has a retention time equal to the standard 4,4′-dichlorobiphenyl. The yield of this pure component is low mainly because there is a net overall equal removal of the undesired contaminants with the desired 4,4′-dichlorobiphenyl by recrystallization. The characteristic radiochromatogram of this purified material is shown in Fig. 10.

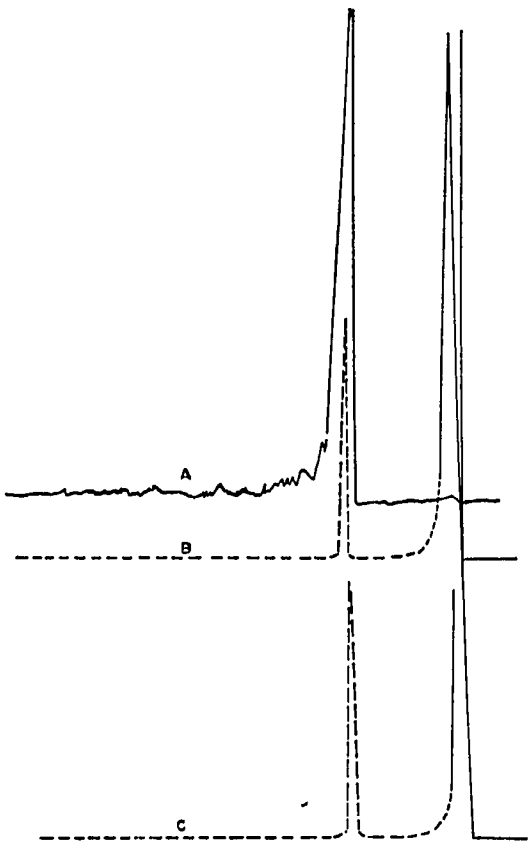


Fig. 10. Radiochromatogram of the purified ^{14}C-labeled 4,4′-dichlorobiphenyl synthesized by the Ullman reaction. (A) Distribution of radioactivity for the purified ^{14}C-labeled 4,4′-dichlorobiphenyl as determined by the gas flow proportional counter; range, 20 K; time constant, 2; discriminator mode, A-α. Analytical conditions, as described for Fig. 6. (B) Flame ionization detector response for the purified ^{14}C-labeled 4,4′-dichlorobiphenyl. (C) Flame ionization detector response for standard unlabeled 4,4′-dichlorobiphenyl.

As shown up to this point of the discussion, GLC coupled with EI mass spectrometry is a very powerful analytical tool for investigating the isomeric purity of PCB's from various sources. By using this same type of information, one can more fully understand the reaction mechanism of the Ullman synthesis.

Because of the lack of a thorough understanding concerning the mechanism of reaction of the Ullman synthesis, we investigated the possible sources of some of the undesirable contaminants of this reaction. Since, as described earlier, we are very much interested in the *ortho–ortho* interaction of chlorine atoms of specific PCB's, we attempted to prepare ^{14}C-labeled 2,2'-dichlorobiphenyl using freshly prepared copper dust as described for the previously described ^{14}C-labeled 4,4'-dichlorobiphenyl. Without any prior removal of components from the crude reaction mixture, we analyzed the possible source of various contaminants.

The first source of possible contaminants may arise from the specific chloroaniline isomer. ^{14}C-Labeled 1-iodo-2-chlorobenzene was prepared as described earlier. The ^{14}C-labeled 1-iodo-2-chlorobenzene was then analyzed by GLC and by GLC coupled with mass spectrometry to determine the presence of 1-iodo-3-chlorobenzene and 1-iodo-4-chlorobenzene.

As indicated by the results shown in Table IV, only 1-iodo-2-chlorobenzene was formed during the initial reaction. Furthermore, some of this same ^{14}C-labeled 2-chloroaniline hydrochloride and unlabeled 2-chloroaniline hydrochloride was used to prepare the pure ^{14}C-labeled 2-chlorobiphenyl. There were not any detectable isomeric contaminants derived from the ^{14}C-labeled 2-chloroaniline. Therefore, the contaminants described for the ^{14}C-labeled 4,4'-dichlorobiphenyl and the presently discussed ^{14}C-labeled 2,2'-dichlorobiphenyl were formed during the Ullman reaction.

TABLE IV

GLC OF ISOMERIC IODOCHLOROBENZENES

No.	*Sample*	*Relative retention time**	*Comments*
1	Standard 1-iodo-2-chlorobenzene	1.00	Single component
2	Standard 1-iodo-3-chlorobenzene	0.83	Single component
3	Standard 1-iodo-4-chlorobenzene	0.83	Single component
4	^{14}C-Labeled sample prepared from 2-chloro-aniline (^{14}C-labeled 1-iodo-2-chlorobenzene)	1.00	Single component by radioactivity and mass (FID)
5	Sample No. 4 spiked with std. 1-iodo-3-chlorobenzene	(a) 0.83 (b) 1.00	Two components
6	Sample No. 4 spiked with std. 1-iodo-4-chlorobenzene	(a) 0.83 (b) 1.00	Two components
7	Sample No. 4 spiked with std. 1-iodo-2-chlorobenzene	1.00	Single component

* 130° isothermal using 3% cyclohexanedimethanol succinate on 100–120 mesh Gas-Chrom Q, stainless-steel column 4 m × 0.2 cm I.D.

Analysis of the crude ^{14}C-labeled 2,2'-dichlorobiphenyl reaction mixture prior to analysis by mass spectrometry indicates numerous labeled components similar to those described for the ^{14}C-labeled 4,4'-dichlorobiphenyl. At least nine separate radiolabeled components (Fig. 11B) are formed during the Ullman reaction. As indicated by the results of the chromatogram (Fig. 11C), the ^{14}C-labeled 2,2'-dichlorobiphenyl seems to be the major component.

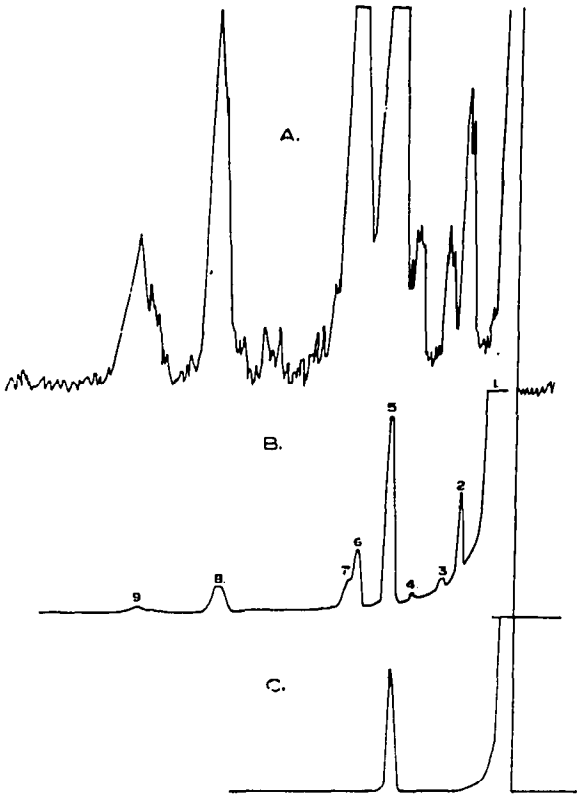


Fig. 11. Radiochromatogram of the Ullman reaction mixture for ^{14}C-labeled 2,2′-dichlorobiphenyl. Analytical conditions were as described for Fig. 6 except that the column was 3% OV-1, with the column temperature being 140° isothermal, the proportional counter radioactivity detection range being 2 K, and the time constant 2. (A) Radioactivity distribution of the crude Ullman reaction mixture of ^{14}C-labeled 2,2′-dichlorobiphenyl, as monitored by the gas flow proportional counter. (B) Flame ionization detector response of the crude ^{14}C-labeled 2,2′-dichlorobiphenyl reaction. (C) Flame ionization detector response for standard 2,2′-dichlorobiphenyl.

Retention indices for some of the contaminants of the ^{14}C-labeled 2,2′-dichlorobiphenyl reaction mixture were determined using 3% cyclohexanedimethanol succinate as described earlier[21]. As shown by Fig. 11B, component 5 is the labeled 2,2′-dichlorobiphenyl with a retention index of 2265; component 6 has a retention index of 2367; component 7 one of 2395; component 8 one of 2515; and component 9 (Fig. 11B) has a retention index of 2595 on cyclohexanedimethanol succinate.

As seen earlier for the ^{14}C-labeled 4,4′-dichlorobiphenyl reaction mixture, during the reaction of the pure isomeric iodochlorobenzene with activated copper dust, there is a general randomization of chlorine atoms to produce other isomeric dichlorobiphenyls.

Fig. 12A is the reconstructed chromatogram of the ^{14}C-labeled reaction mixture derived from ^{14}C-labeled 1-iodo-2-chlorobenzene with the Ullman procedure. Using the analytical conditions as described earlier for Fig. 7A, there are at least three dichlorobiphenyl isomers, one trichlorobiphenyl isomer as determined by the limited

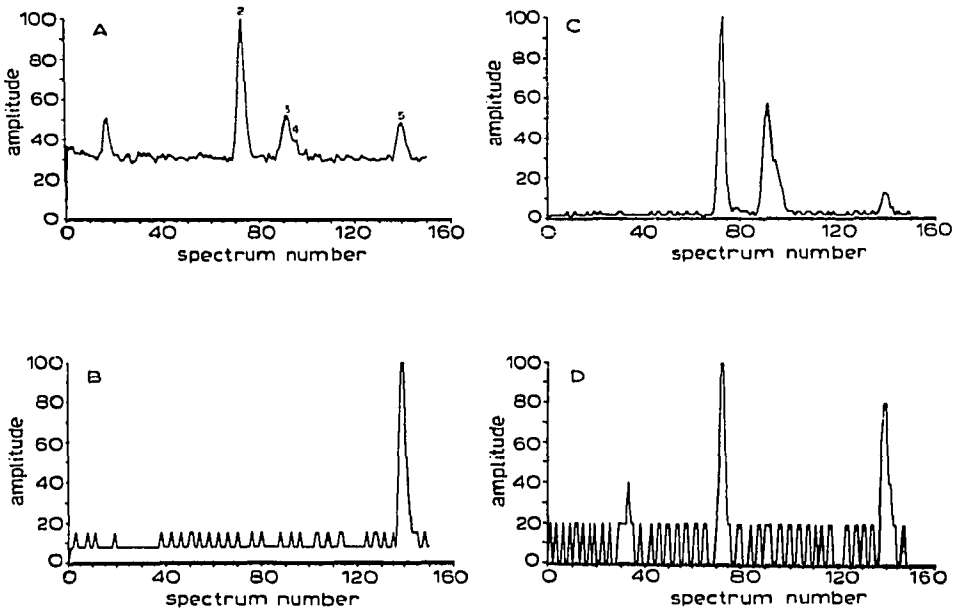


Fig. 12. (A) Reconstructed gas chromatogram of the Ullman reaction mixture for the preparation of ^{14}C-labeled 2,2′-dichlorobiphenyl. This reaction was carried out as described for the ^{14}C-labeled 4,4′-dichlorobiphenyl with the analytical conditions as discussed for Fig. 7A. (B) Limited mass search *m/e* 256–258. (C) Limited mass search *m/e* 222–224. (D) Limited mass search *m/e* 188.

mass search (Figs. 12B–D) and a very low amount of the reduction product. The major component is the ^{14}C-labeled 2,2′-dichlorobiphenyl (spectra 69–78). As in the case of other 2,2′-dichlorobiphenyls, the EI mass spectrum of the 2,2′-dichlorobiphenyls exhibits a very characteristic $(M - Cl)^+$ ion at *m/e* 187. The two additional isomeric dichlorobiphenyls present in this complex mixture have retention times relative to 2,2′-chlorobiphenyl of 1.24 and 1.31, respectively. Neither of these isomeric dichlorobiphenyls (spectra 86–97) exhibit the very abundant and characteristic $(M - Cl)^+$ ion at *m/e* 187 as seen for the 2,2′-dichlorobiphenyl. The major trichlorobiphenyl contaminant (spectra 135–144) in Fig. 12A does possess the very abundant $(M - Cl)^+$ ion at *m/e* 221 and has a retention time of 1.90 relative to that of ^{14}C-labeled 2,2′-dichlorobiphenyl.

By controlling the time of initiation of the ionizer and in some cases by concentration of sample, one may further distinguish the presence of other trace components. Using the same chromatographic conditions as described for Fig. 12, upon initiation of the ionizer and computer 4 min 30 sec after injection, one obtains a chromatogram as shown in Fig. 13. Peaks 1 and 2 of Fig. 13A represent the isomeric dichlorobiphenyls with retention times relative to the 2,2′-dichlorobiphenyl of 1.24 and 1.31, respectively. The limited mass search *m/e* 256–258 indicates that component 3 (Fig. 13A) is the major trichlorobiphenyl with a relative retention of 1.90.

In order to investigate further the presence of other reaction products generated during the Ullman reaction, the sample was concentrated so that approximately 4 μg of material was applied to the gas chromatographic column with the ionizer being

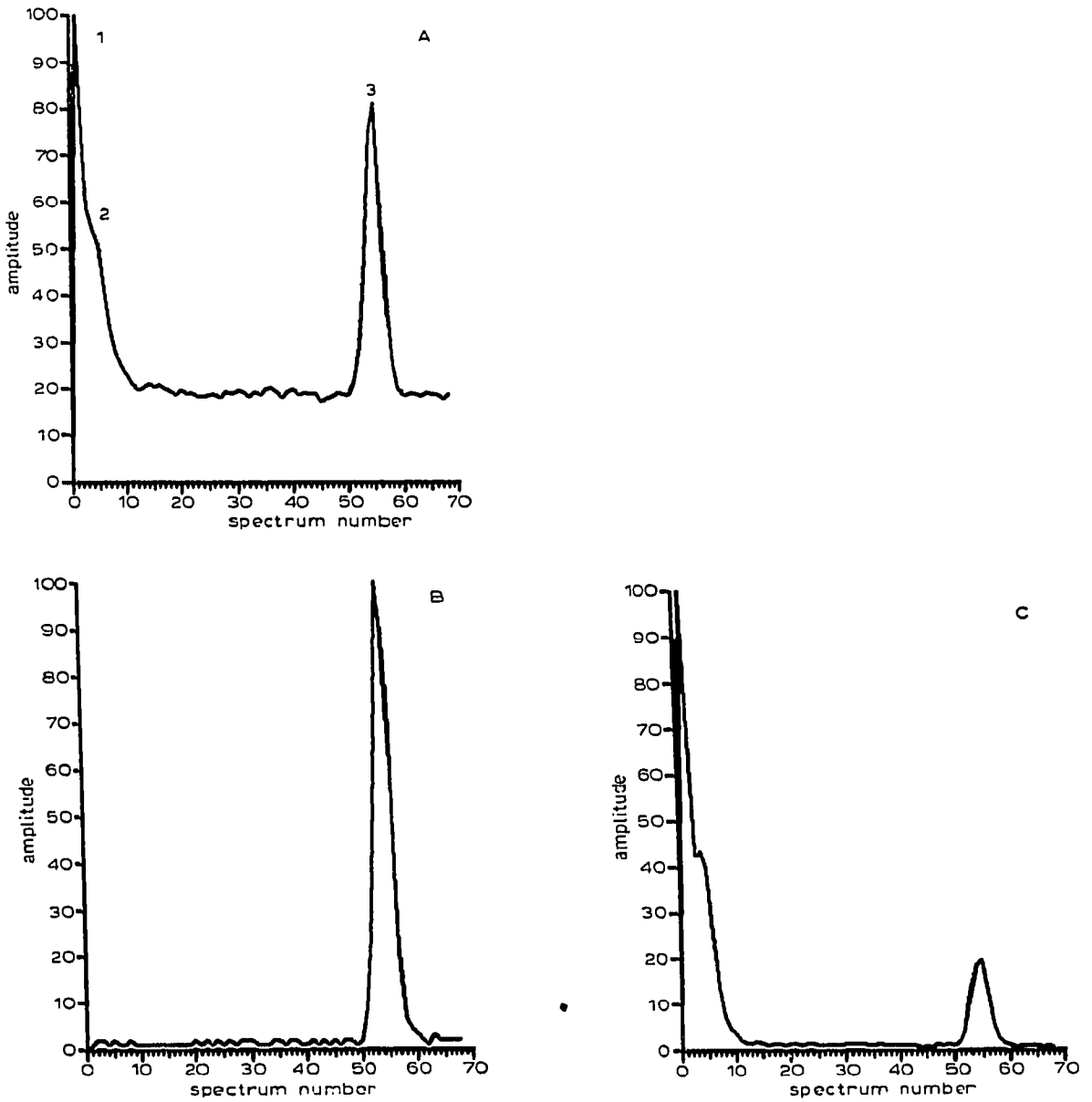


Fig. 13. (A) Reconstructed gas chromatogram of the crude reaction mixture of ^{14}C-labeled 2,2′-dichlorobiphenyl prepared by the Ullman reaction. Analytical conditions as described for Fig. 7A except that the ionizer was initiated 4 min 30 sec after injection of the sample. (B) Limited mass search *m/e* 256–258. (C) Limited mass search *m/e* 222–224.

initiated 5 min 45 sec after injection with the same chromatographic conditions as described for Figs. 7A, 12A and 13A. In Fig. 14A, peak 1 (spectra 8–18) is the major trichlorobiphenyl with a retention time of 1.90 relative to the 2,2′-dichlorobiphenyl and with a very abundant $(M - Cl)^+$ ion at *m/e* 221 as indicated by the limited mass

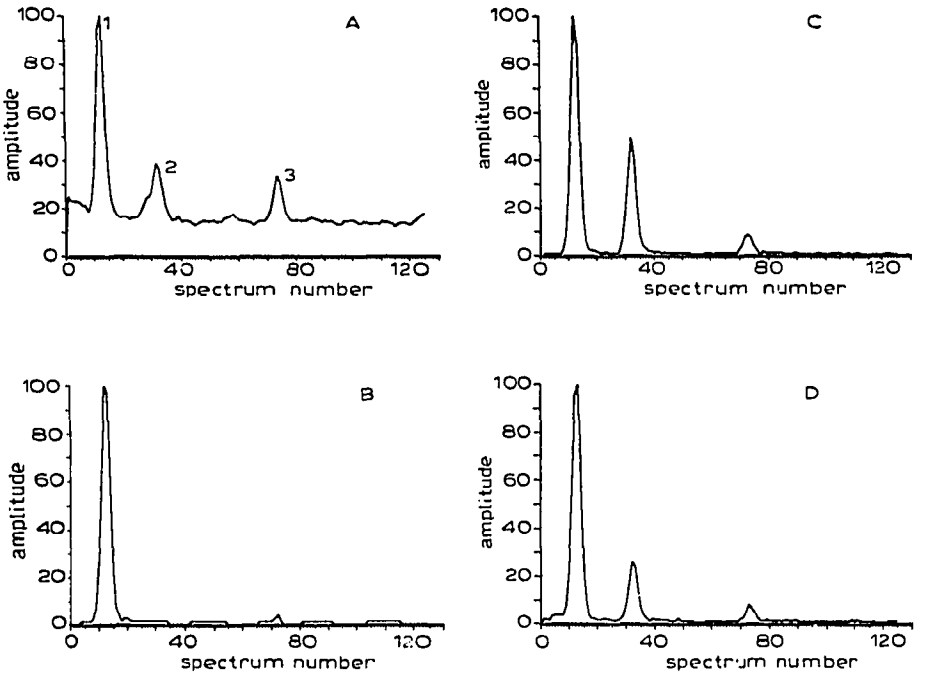


Fig. 14. (A) Reconstructed gas chromatogram of the crude ^{14}C-labeled 2,2'-dichlorobiphenyl mixtures analyzed by GLC–EI mass spectrometry. Analytical conditions as described for Fig. 7A except that the ionizer was initiated 5 min 45 sec after injection. (B) Limited mass search *m/e* 221. (C) Limited mass search *m/e* 256–258. (D) Limited mass search *m/e* 185–187.

search in Fig. 14B. Components 2 and 3 of this chromatogram (Fig. 14A) are also isomeric trichlorobiphenyls but do not have the abundant *m/e* 221 ion. As expected, all three of these trichlorobiphenyls produce the $(M - Cl_2)^+$ ion in the region *m/e* 185–187 (Fig. 14D).

In concluding the discussion on the isomeric contaminants present in the Ullman synthesis of ^{14}C-labeled 2,2'-dichlorobiphenyl, this report has described the presence in addition to the desired ^{14}C-labeled 2,2'-dichlorobiphenyl of at least: (a) two other isomeric dichlorobiphenyls which do not possess 2,2'-chlorine substitution; (b) a major trichlorobiphenyl which does possess 2,2'-chlorine substitution; and (c) at least two trichlorobiphenyls which do not possess 2,2'-chlorine substitution. Representative EI mass spectra of these ^{14}C-labeled components derived from the reaction of pure ^{14}C-labeled 1-iodo-2-chlorobenzene and freshly prepared copper dust are shown in Figs. 15A–D.

The Ullman reaction does not synthesize a single polychlorinated biphenyl isomer but it produces isomeric dichloro- and trichlorobiphenyls from the specific iodomonochlorobenzene. By repetitive discarding of this complex mixture as accomplished by recrystallization, one may obtain a single component only after great sacrifice in loss of the desired compound.

With reference to the mechanism of reaction for the Ullman syntheses as described in this report, it may be concluded that at least four types of reaction take place in the presence of a pure isomeric iodochlorobenzene and catalytic copper dust:

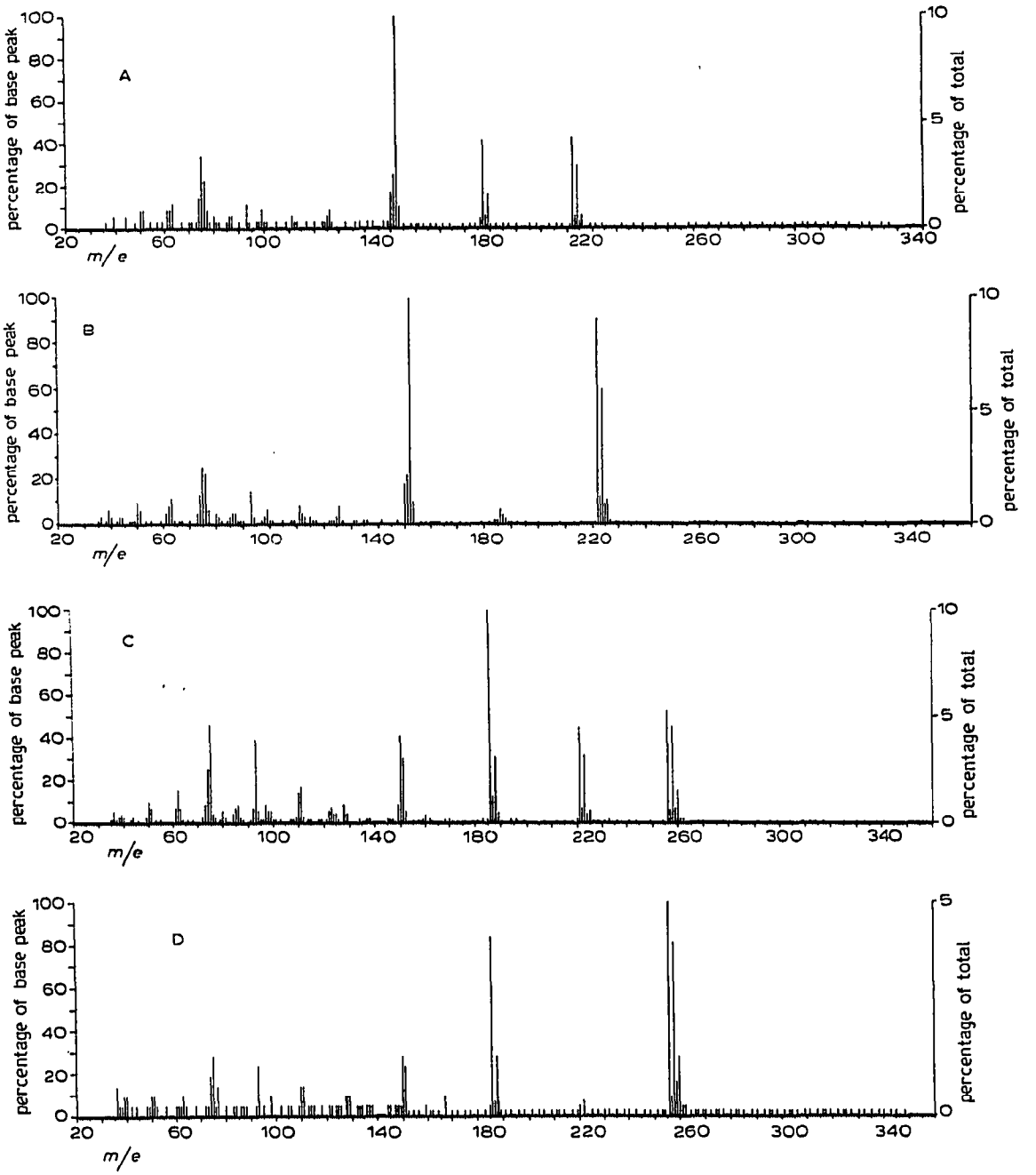


Fig. 15. (A) 70-eV EI mass spectrum of ^{14}C-labeled 2,2′-dichlorobiphenyl (spectrum 73–66; Fig. 12A). (B) Isomeric dichlorobiphenyl contaminant of the ^{14}C-labeled 2,2′-dichlorobiphenyl synthesis which does not possess 2,2′-chlorine substitution (spectrum 1–13; Fig. 13A). (C) Major trichlorobiphenyl isomeric contaminant of the Ullman synthesis of ^{14}C-labeled 2,2′-dichlorobiphenyl (spectrum 13–6; Fig. 14A). (D) Isomeric trichlorobiphenyl contaminant of the 2,2′-dichlorobiphenyl synthesis which does not possess 2,2′-chlorine substitution (spectrum 32–24; Fig. 14A).

(1) Reduction to yield the monochlorobiphenyl is a reaction which can be minimized by the use of freshly prepared copper dust. (2) Using freshly prepared copper dust, the coupling of the specific isomeric iodochlorobenzene to yield either the 4,4'-dichlorobiphenyl or the 2,2'-dichlorobiphenyl is the major reaction. (3) A very abundant reaction that takes place results in the randomization of chlorine atoms to yield undesirable isomeric contaminants; at least two explanations may be applied to this randomization of chlorine atoms in the Ullman reaction; upon reaction of the iodochlorobenzene with copper, two types of reactive species may be formed: (a) one possible reactive intermediate would be a chlorobenzyne, in which the triple bond character would be distributed throughout the aromatic ring; coupling of the benzyne species with other iodochloro- or chlorobenzenes would generate the non-directed or randomized chlorine isomers; (b) a second possible reactive species would be a free radical formed during removal of the iodine atom by copper dust from the ring to yield a net randomized chlorobiphenyl upon coupling with another aromatic molecule. (4) Another very abundant reaction that takes place during the Ullman synthesis is chlorine transfer. As shown for both ^{14}C-labeled dichlorobiphenyl syntheses, beginning with a pure isomer of the iodomonochlorobenzene, major contaminants of the reaction are trichlorobiphenyls. The increased number of chlorine atoms per molecule of biphenyl may have been caused by the generation of chlorine free radicals on the copper surface.

This report has described the advantages of the utilization of GLC coupled with mass spectrometry for the characterization and isomeric differentiation of polychlorinated biphenyls. By computer limited mass searches of specific data, one is able to identify and differentiate many chlorinated biphenyl components of a gas chromatogram. Furthermore, by the indepth characterization of the Ullman reaction products coupled with the preparation of ^{14}C-labeled PCB's, one may conclude that the conditions of the Ullman reaction do not yield one directed and concerted isomeric product but instead yield a very complex isomeric mixture. Care must be exercised in choosing the types of organic synthetic reaction for the preparation of PCB's that will be used for biological testing. In addition, one must very scrupulously analyze even the pure isomeric PCB's for trace impurities before proceeding to introduce this specific PCB into a biological system. Only after numerous investigations have been carried out with pure well defined isomeric PCB's will one be able to fully assess the biological activity of individual components and the potential health hazards produced by these widely distributed environmental agents.

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