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Report

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PCBs | Regulatory History and Analytical Problems

Synthesis of polychlorinated biphenyls (PCBs) was first described by Schmidt and Schultz (*1*) in 1881, but the industrial application of that discovery was not fully developed until 50 years later by the Swan Corporation. Commercial introduction of PCBs as industrial chemicals for use as nonflammable oils, notably in connection with electrical transformers, condensers, and paint, was begun in 1930. Chlorination of the parent moiety, biphenyl, results in a series of chlorinated products which exhibit unique characteristics of both thermal and chemical stability. These mixtures of PCBs (rather than individual isomers) quickly gained wide acceptance as industrial products where nonflammability and heat-resistance properties were highly desired. PCBs were marketed and used by manufacturers in products under various trade names: Aroclor, Clophen, Phenoclor, Kanechlor, and Phyralene, to name just a few of the major product lines.

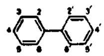
In theory, the total number of possible products resulting from chlorination of biphenyl is 209 (Figure 1), compared to 135 isomers for the closely related polychlorinated dibenzofurans (PCDFs) present as impurities in commercial PCBs. The physical properties of such mixtures are quite different from those of individual species. Aroclors will not react with acids, alkali, or water under normal or even vigorous conditions. Boiling points for Aroclors range from 278 °C for 21% chlorinated product to about 420 °C for 68% chlorinated product. PCBs can be distilled at atmospheric pressure without carbonization or decomposition. They are insoluble in aqueous media, but soluble in hydrocarbon solvents.

Regulatory History

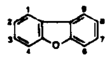
From the initial introduction of PCBs as industrial chemicals in 1930, their widespread popularity in uncontrolled applications (hundreds of millions of pounds) over the ensuing 40 years finally resulted in their becoming a persistent and ubiquitous environmental contaminant. In 1966, Jensen (*2*), a Swedish scientist, was the first to draw attention to the fact that PCBs were found in fish and birds. By late 1971, it had become glaringly obvious that contamination of the environment had led to global contamination of wildlife (*3*). In essence, PCBs along with DDE had become the most abundant of the chlorinated aromatic pollutants in the ecosystem. The most serious consequence of the widespread contamination of the environment with PCBs was the indirect contamination of certain foods. PCBs were found in human adipose tissue and human milk.

In 1968 an unfortunate accident in Japan (*4*) was a sneak preview of several similar but less serious accidents that occurred in the U.S. during the following decade. Rice oil became contaminated when PCBs leaked from a heat exchanger. The so-called "Yusho" poisoning incident resulted when victims consumed the contaminated rice oil. A whole spectrum of adverse symptoms was observed, e.g., chloracne, discoloration of the gums and nailbeds, swelling of the joints, waxy secretions of the glands in the eyelids, as well as more general effects such as lethargy and joint pain. This single incident served as the catalyst to focus much greater attention on the problem of PCB contamination of foods.

At that point, the U.S. Food and Drug Administration (FDA) conducted a national survey to determine the extent and levels to which PCBs might find their way into the food chain via indirect use of PCB-contaminated animal feed, industrial and environmental sources, and the use of paper food-packaging materials con-



Polychlorinated Biphenyls (PCBs)



Polychlorinated Dibenzofurans (PCDFs)

Chlorine Substitution	PCBs	PCDFs
Mono-	3	4
Di-	12	16
Tri-	24	28
Tetra-	42	38
Penta-	46	28
Hexa-	42	16
Hepta-	24	4
Octa-	12	1
Nona-	3	—
Deca-	1	—
Totals –	209	135

Figure 1. Number of possible isomers of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs)

taining PCBs. In 1972, this survey culminated in a "Notice of Proposed Rule Making" (5) to limit the level of PCBs in foods containing unavoidable PCB residues from environmental or industrial sources. Analysis of animal feed indicated less than 5% of the samples contained PCBs (0–0.6 ppm). Isolated accidents, however, had occurred in pasteurization equipment, where PCBs from heat exchanger fluids had directly contaminated animal feed and subsequently poultry and eggs intended for human consumption. The use of PCB-containing coatings on the inner walls of silos had also resulted in the contamination of silage, which had in turn caused PCB residues to appear in the milk of dairy cows (6).

In a survey on food packaging, 67% of the packaging samples contained PCBs, with the highest level observed being 338 ppm. Of these samples, only 19% of the actual food contained in the packaging contained PCBs, with a maximum level of 0.1 ppm. In the case of packaged infant food cereals, 75% of the samples contained PCBs, with an average concentration of 0.3 ppm; the maximum found was 1 ppm. At the time of this survey, knowledge of the toxicological effects of PCBs was somewhat limited. The FDA concluded that it would be prudent to reduce human low-level exposure to PCBs by limiting the ways in which PCBs might enter the food chain, and to limit the levels of PCBs in food containing unavoidable PCB residues from environmental or industrial sources by establishing temporary tolerances (Table I) until such time as additional information warranted change (7).

The temporary tolerances established in 1973 were a result of a vigorous evaluation of the data base on PCBs prevailing at that time. Concern about the ubiquitous distribution of PCBs in the ecosystem and the "Yusho" accident led to a massive scientific probe into the toxicity of PCBs, as well as their reported presence in most wildlife (8). In 1975, the Environmental Protection Agency (EPA) sponsored a national conference on PCBs, at which the FDA announced it had initiated a review of the appropriateness of the 1973 temporary tolerances. After reviewing the wealth of data generated by the scientific community and government on PCBs, FDA proposed to reduce the temporary tolerances (9) for unavoidable residues of PCBs in several classes of food. More than 100 comments on this proposal were received from interested parties. In considering these comments, the FDA commissioner issued a final order reducing PCB tolerances (Table I) (10).

Table I. FDA Tolerances for Polychlorinated Biphenyls in Several Classes of Food

Product	Concentration (ppm) 1973	Concentration (ppm) 1979–present
Milk and dairy products (fat basis)	2.5	1.5
Poultry (fat basis)	3.0	3.0
Eggs	0.3	0.3
Fish and shellfish (edible portion)	5.0	5.0*
Infant and junior foods	0.2	0.2
Feed for food-producing animals	0.5	0.2
Animal feed components including fish meal and other byproducts of marine origin	5.0	2.0
Paper food-packaging materials intended for or used with human food	10.0	10.0

* A regulation establishing a new level of 2 ppm was promulgated but was stayed on Oct. 5, 1979, until further notice.

Since the food and feed industries were particularly vulnerable to accidental contamination from PCBs, EPA, which has regulatory control of PCBs and other toxic substances under the Toxic Substance Control Act of 1976, has issued rules governing their continued use. As of Nov. 1, 1979, the use of PCBs in new heat transfer systems in plants manufacturing or processing food, drugs, and cosmetics was no longer authorized. The EPA rule, however, did permit the continued use of PCBs in electromagnets, transformers, heat transfer and hydraulic systems until July 1, 1984. Issuance of an interagency alert notice was prepared under the sponsorship of EPA to urge voluntary removal of equipment and its replacement with non-PCB units to prevent food contamination (11).

Toxicity

Evaluation of the toxicity of PCBs has been complicated by the heterogeneity of the commercial preparations and the analytical difficulties in separation, identification and quantitation. Acute oral LD_{50} values of PCBs in mammals vary from 2–10 g/kg, with an apparent increase in mammalian toxicity with a decrease in chlorine content. In humans, chronic ingestion of small amounts (10 mg/kg) for more than 50 days causes chloracne. The fear that prolonged, continuous exposure to low doses might well result in serious human health problems has promoted a massive scientific inquiry into the toxicological and biological effects of PCBs. A recent study, involving the feeding of male broiler chickens (12) low levels of Aroclor 1254 from hatching to eight weeks of age, demonstrated marked reduction in growth with concurrent accumulation of PCBs in all tissues. Fukano et al. (13) have illustrated the accumulation of PCBs in human fat for both males and females from Tokyo within the range 1–2 ppm, while blood samples contained 1–5 ppb PCBs. Recently, the eloquent demonstration by Poland (14) that the toxicity of certain PCB congeners together with other polycyclic aromatics (PCDFs, azobenzenes, and TCDD) paralleled their ability to compete (in vitro) for specific binding sites in the cytosol carrier proteins has provided a useful tool for structure-activity and mechanistic studies.

To complicate the issues, the wealth of data concerning the metabolism of PCBs has given rise to another set of analytical problems. It is now clearly established that the major metabolic route of PCBs is hydroxylation. Metabolism in rat microsomal systems (15) of selected tri-, tetra-, and pentachlorobiphenyls resulted primarily in the formation of monohydroxylated PCBs with the number of metabolites decreasing with increasing degree of chlorination. Similar studies with PCBs in the rhesus monkey (16) have confirmed this pathway, and also demonstrated dechlorination and arene oxide formation. Four lactating Holstein cows fed single doses of Aroclors 1242 and 1254 (1.5 g) excreted in their milk 10 monohydroxy PCBs and four monohydroxy PCBs, respectively (17). Jensen et al. (18) have also reported in a study of seal blubber that methyl sulfone metabolism is clearly evident. The seal blubber contained approximately 150 ppm total PCBs, with 16 ppm of total PCB sulfones. The concentration of PCBs stored in the fat tissue increases and is then transferred at increasing concentrations to the food chain.

Analytical Considerations

It should now be clear that any attempt to extract, separate, identify, and quantify a mixture of PCBs re-

quires knowledge of three major problem areas:

Composition. In spite of theoretical calculations establishing 209 possible congeners, Welti (19) has demonstrated experimentally via capillary chromatography that not all possible compounds are present in Aroclor 1254. Of the 69 eluting peak profiles observed, 19 represented major components while 50 represented minor contributions to the mixture. Certain positions on the biphenyl nucleus are not favored for chlorine substitution; 2-, 4-, and 6- substitutions are rare, and the occurrence of 3-, 3,5-, and 2,3- isomers is likewise infrequent. Parallel studies using packed columns have shown elution profiles with fewer than 20 discernible peaks. The complications induced by analyzing mixtures of PCBs rather than any single specific isomer have contributed a serious impediment to quantitation, since most analyses ultimately involve estimation of compounds in which the degree of chlorination can run from mono- to deca-, i.e., a range of molecular weights (188–494) with varying chemical and physical properties.

Interfering Organochlorine Pesticides. The initial confirmation of the occurrence of PCB residues in wildlife by Jensen (2) was reported after repeated and frequent encounters of similar elution patterns or profiles while analyzing for DDT and other chlorinated pesticides in general. Many earlier reported residue analyses failed to recognize this PCB in-

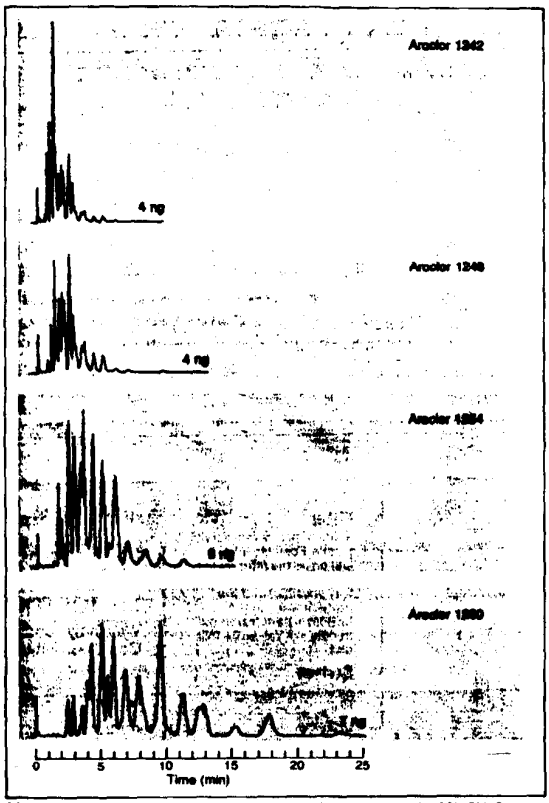


Figure 2. Electron capture responses of various Aroclor standards: 2% OV101, 6 ft × 2 mm, 200 °C

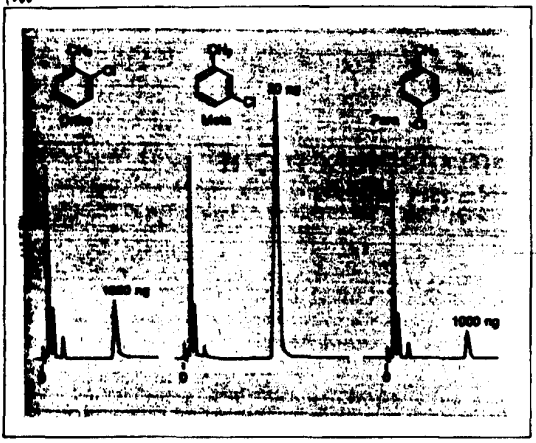


Figure 3. Electron capture responses to *ortho*-, *meta*-, and *para*-chlorotoluene: 2% OV101, 6 ft × 2 mm, 70 °C, attenuation 64 × 10^{-11} amps

terference, which must have led to an overestimation of DDT in the environment. After this historical breakthrough in 1966, the roles were suddenly reversed and the important PCB residue analyses were then deemed to have interferences by a whole host of common and ubiquitous organochlorine pesticides. Methodologies were then devised to efficiently separate PCBs from such organochlorine pesticides as DDT and Toxaphene, Heptachlorepoxide, Lindane, and Dieldrin. The stability of PCBs to treatment with acids and alkali made it possible in some procedures to either destroy or alter one or more of the interfering pesticides. On the other hand, greater attention has been paid to the use of various columns (e.g. silicic acid–celite and Florisil) and solvents (mixtures of acetonitrile, hexane, and methylene chloride) to separate PCBs from the other chlorinated pesticides (20). Masumoto (21) has pointed out, however, that such procedures can be flawed by variable column preparation and hence variable recoveries. Additionally, those congeners with the lowest chlorination were held on the silicic column and could only be eluted by a more polar solvent than petroleum ether, leading to lower recoveries for the less chlorinated Aroclors. The analyst must choose appropriate analytical procedures, depending on which interferences are present.

Metabolism. The elution patterns or profiles observed by electron capture (EC) gas chromatography for PCB residues from biological samples (22) very often do not resemble the profile for one of the PCB standards selected for quantitation. The presence of metabolites and degradation products is likely to be primarily responsible for this experimental observation. Dechlorination, arene oxide formation, and hydroxylation have been identified as metabolic pathways for PCBs (23). Dechlorination to lesser chlorinated congeners will tend to contribute to the production of a profile perhaps suggestive of a less chlorinated Aroclor and will lead to confusion in the choice of the correct Aroclor standard for quantitation by EC. More serious by far is the removal from the observed PCB residue profile of congeners that have undergone

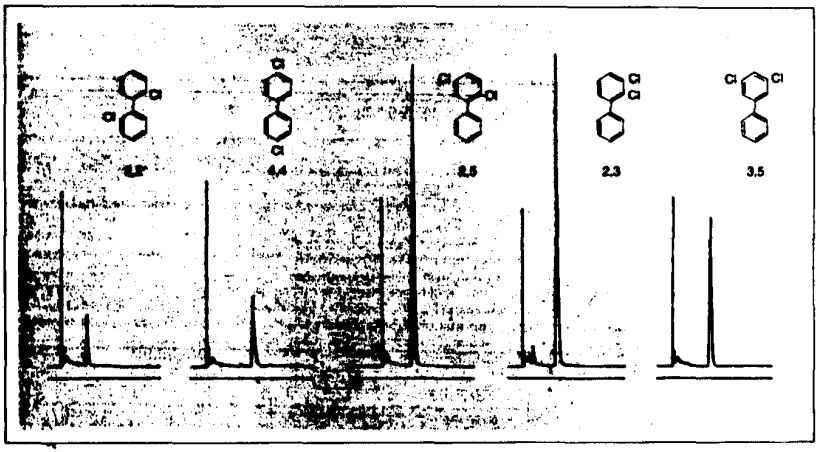


Figure 4. Electron capture responses for 1 ng injected of selected PCBs: 2% OV101, 6 ft × 2 mm, 180 °C, attenuation 16 × 10^{-11} amps

metabolic hydroxylation. Such chlorobiphenylols do not exhibit the same elution characteristics as the parent substances, and therefore do not coelute with the solvent systems generally employed.

Metabolic processes can thus confuse the assignment of the original source of the PCB contamination and hence the correct choice of a suitable standard for quantitation. Such PCB residue profiles are further complicated by the fact that extraction and cleanup can alter the composition of mixtures because of the unique properties of these congeners.

Approaches to Quantitation

The problems encountered in the quantitation of PCB residues have focused the attention of the analytical chemist on methods by which the concentration of PCBs is related to the estimation of a single species. Such attempts have included: carbon–skeleton GC, where PCBs are reduced on-column (5% Pt at 180 °C with H_2 as carrier gas) to biphenyl (*24*); perchlorination of PCBs by antimony pentachloride to yield decachlorobiphenyl (*25*); and microcoulometric determination of the total chlorine content as HCl (*26*). These methodologies have been developed because EC-GC detector response is disproportionate, i.e., highly dependent on the degree of chlorination of the biphenyl nucleus. EC detector response has been shown to vary as much as two to four orders of magnitude between monochloro and polychloro species (*27*). The proliferation of analytical approaches to PCB analysis has resulted mainly from the existence of this disproportionality factor (*28*). This issue has overshadowed other problems, such as interferences resulting from the presence of other organochloride pesticides and PCB metabolites.

Of prime importance when using EC as the detection system for chlorinated aromatic species such as the PCBs is the problem of disproportionate responses. At the present time, Aroclor standards have been characterized by EC (Figure 2) and are employed as reference materials. The closest matching profile is used as a standard for quantitation of environmental samples. A good example of disproportionality of response by EC is that exhibited by the isomeric monochlorotoluenes (Figure 3), in which differences of several orders of magnitude were observed. Although this case is a relatively dramatic example, the prevailing situation within the congeners of the PCB family has been found to be similar but somewhat less pronounced. The EC responses to 1 ng of five selected dichlorobiphenyls under similar conditions are illustrated in Figure 4. Depending on the location of the chlorine atoms within the biphenyl nucleus, varying detector responses were observed. This disturbing disproportionality has been the main criticism of PCB analysis with EC.

The analytical pitfalls created by the lack of well-defined standards when dealing with mixtures has ultimately led to the increased application of mass spectrometry in its various forms to assist in resolving difficult problems concerning identification and quantitation of these systems. A novel approach to routine quantitative analysis by chemical ionization mass spectrometry is described in the research section of this issue (*29*).

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