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Advances in Exposure, Health and Environmental Effects Studies of PCBs

ICAIR Life Systems, Inc.
Cleveland, Ohio

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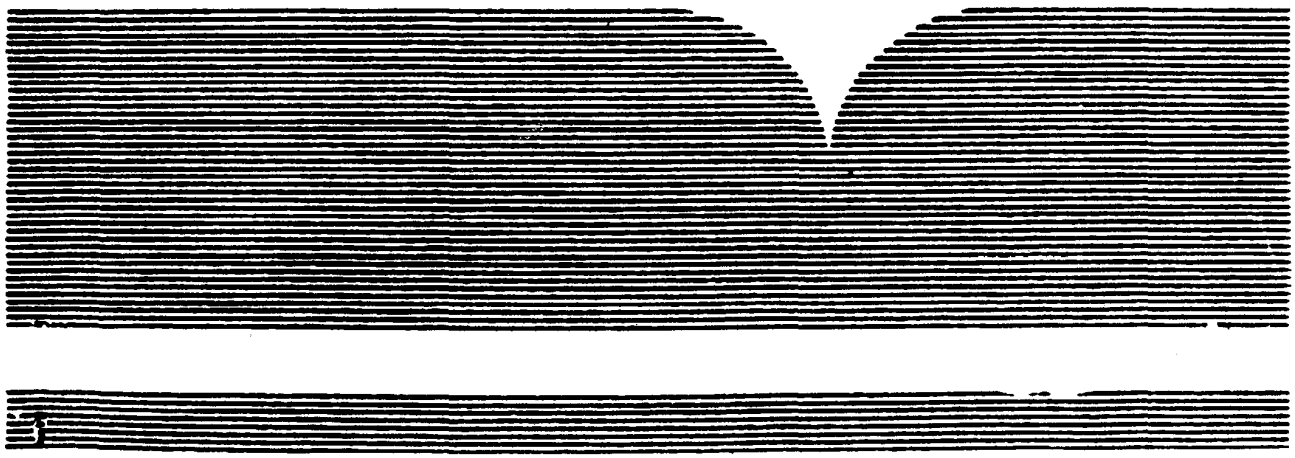

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SYMPOSIUM PROCEEDINGS

May 12-13, 1982

Prepared Under Contract No. 68-01-6554
Project 1247
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by

ICAIR
Life Systems, Inc.
Cleveland, OH 44122

for

Office of Toxic Substances
Health and Environmental Review Division
U.S. Environmental Protection Agency
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Analytical Methodologies;
Exposure Studies: Environmental Residues and Bioaccumulation;
Exposure Studies: Industrial Processes;
Health Effects: Epidemiology;
Health Effects: Laboratory Studies;
Environmental Effects; and
Risk Assessment

Each contributed paper includes a significant bibliography of cited and related scientific literature references for those seeking greater detail.

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DISCLAIMER

Although this Proceedings Document reports the presentations and discussion that occurred in the PCB Symposium funded by the United States Environmental Protection Agency through Contract No. 68-01-6554 with Life Systems, Inc., the contents of the Proceedings are the independent views of scientists not associated with EPA. Consequently, the Proceedings Document has not been subjected to the Agency's peer review process. It does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

FOREWORD

Almost anyone who is likely to open this book will know the basic facts about polychlorinated biphenyls (PCBs): first described in the late 19th century, they have been produced on an industrial scale since about 1930. In the subsequent 40 years they have been used extensively in a variety of applications because of their chemical and thermal stability and good dielectric properties. Since the different commercial PCB products are not simple entities but families of compounds, the picture is complicated because individual components of these mixtures differ in their chemical, physical and biological properties.

The initial concern about chlorinated aromatic compounds originated from reports around 1940 of chloracne and liver changes in workers who came in contact with these materials during production or handling. Worldwide attention was not given to PCBs, however, until 1966 when the journal, New Scientist, reported on Soven Jensen's finding of PCBs in pike from Swedish lakes, in the human brain and in an eagle which was found dead in the Stockholm area. During the years that followed, PCBs were found in environmental samples in several parts of the world. It became evident that these persistent and bioaccumulating compounds were ubiquitously distributed; biological material from polar regions and other uninhabited and remote areas contained traces of PCBs.

The U.S. manufacturers responded to these findings by limiting sales of PCBs to uses in enclosed systems only. The presence of PCBs in some isolated environmental samples from Sweden might have remained an analytical curiosity for some time had not Rachael Carson's book, "Silent Spring", alerted environmental scientists - and the general public - some years before to the possible danger of organochlorine compounds.

In 1968 accidental ingestion of PCBs through contaminated rice oil revealed a number of toxic effects of PCBs in a group of Japanese people. This incident also established placental transfer of these compounds and persistence in the milk of contaminated mothers. Comparable effects were observed in the 1979 PCBs poisoning incident in Taiwan.

In the early 1970s, it was also shown that humans were directly exposed to PCBs from such diverse sources as food packaging materials and carbonless copying paper. The same time period also saw the beginning of intensive scientific efforts to understand the route of exposure and the fate and effects of PCBs.

Long term exposures at low levels were studied and the phenomena of bioaccumulation and food-chain magnification were also investigated. Prior to studies with PCBs, little was known on photochemical behavior and metabolic patterns of chloroaromatic compounds.

PCBs production and import is either forbidden or limited in most industrialised countries. However, most of the total PCBs produced to date are either still in service or found in dumps and land fills. To avoid further environmental contamination, safe methods for ultimate disposal have to be found.

The PCBs problem will not disappear overnight. There may be emotional reaction and unfounded fear concerning PCBs, but there is also the possibility of real environmental problems with potentially hazardous consequences. We must look at the facts, evaluate them and conduct investigations in areas where additional scientific research is required. A scientifically sound basis for prudent political decisions must be developed. The U.S. Environmental Protection Agency and Life Systems, Inc. are to be commended for organizing this important Symposium.

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University of Bayreuth
Bayreuth, Germany

ACKNOWLEDGEMENTS

The symposium from which this document was prepared, "Recent Advances in Exposure, Health, and Environmental Effects Studies of PCBs," was held May 12-13, 1982 at the Holiday Inn in Bethesda, Maryland. The symposium was organized by ICAIR of Life Systems, Inc. The effort was performed under Contract No. 68-01-6554 from the U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances.

We wish to gratefully acknowledge the administrative services of the following individuals responsible for the program organization, execution, and subsequent preparation of these proceedings: Dr. Irvin Baumel, Dr. William Farland and Mr. H. Glenn Williams of the Office of Pesticides and Toxic Substances, and Mr. Michael Kangas, Ms. Cynthia Patrick, Dr. Roy Reuter, Mr. Greg Schiefer, Ms. Patricia Sweeney and Ms. Cynthia Wesley of ICAIR.

Special appreciation is extended to the symposium participants for the excellent quality of the presentations, the professional manner in which the sessions were conducted, and the stimulating nature of the discussions.

It was the combined efforts of all these individuals which ensured the success of this symposium and the resultant contribution of knowledge to the scientific community.

R. J. Davenport
B. K. Bernard

CONTENTS

continued-

Contents - continued

CHAPTER 1

INTRODUCTION

This publication is a compilation of data presented and discussed during the symposium entitled, "Recent Advances in Exposure, Health and Environmental Effects Studies of PCBs," held May 12-13, 1982 in Bethesda, Maryland. The symposium was organized specifically as an information update for the Environmental Protection Agency's (EPA's) Polychlorinated Biphenyls (PCBs) Program. In more general terms, the symposium provided an opportunity for the exchange of information between PCBs researchers from academia, government agencies, industry and independent research institutions.

The PCBs Program was implemented by the Office of Pesticides and Toxic Substances (OPTS) to carry out the specific requirements of Section 6(e) of the Toxic Substances Control Act (TSCA). Section 6(e) mandates the promulgation of rules by the EPA for the disposal and marking of PCBs, and establishes a timetable for controlling the use of PCBs, and for eliminating the manufacture, processing and distribution of PCBs in commerce.

As part of the continuing update process for acquiring relevant data for the PCBs Program, the OPTS requested that a two-day symposium be undertaken which would provide a forum for a full and free discussion of scientific issues related to PCBs.

Symposium Scope and Objectives

The data presented in the symposium was limited to that which has become available since 1978, including both published data and data from studies that are still in progress. No formal consideration was given the EPA's past or potential regulatory activities concerning PCBs.

The following objectives were established for the symposium:

1. To review, discuss and interpret PCBs scientific data published since 1978 and preliminary PCBs data from ongoing work.

2. To provide for an exchange of views between scientists who are knowledgeable about exposure, health and environmental effects, and human health studies associated with PCBs.

3. To identify research gaps and areas of agreement and controversy.

Symposium Participants

The following individuals participated as the Chairperson, as Speakers or as Discussion Leaders:

Chairperson:

Dr. Otto Hutzinger*
University of Amsterdam
The Netherlands

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Organization of Symposium

In order to achieve the above symposium objectives, the symposium was divided into seven sessions. The session topics, in order of presentation were as follows:

Analytical Methodologies
Exposure Studies: Environmental Residues and Bioaccumulation
Exposure Studies: Industrial Processes
Health Effects: Epidemiology
Health Effects: Laboratory Studies
Environmental Effects
Risk Assessment

Each session consisted of the presentation of papers by one to three speakers, followed by a discussion period. During the discussion period, a dialogue was established between the speakers and the symposium attendees. Questions were answered, and data and the interpretations of data were exchanged.

Each Discussion Leader moderated the discussion period for one or two sessions. Following the symposium, the Discussion Leaders prepared summaries of the discussions from their notes and transcripts made during the symposium.

The chapters that follow are arranged in the order of the seven sessions. Each chapter contains the papers that were presented and the discussion summary, prepared by the Discussion Leader.

The last chapter (Chapter 9) is a summary of the accomplishments and results of the work presented and discussed during the symposium. Although written from the technological viewpoint of a single participant (the symposium Chairperson, Dr. Otto Hutzinger), it provides a needed interpretation of the many facts, recent technical advances and identified data gaps that were presented and discussed during this two-day period.

CHAPTER 2

ANALYTICAL METHODOLOGIES

This chapter reviews certain analytical advances in PCBs detection, identification, and quantification. High resolution gas chromatography is covered in detail, with specific references to capillary column development and supporting 'phase' methodologies, detector systems, and the comparative advantages and limitations of each combination. Problems associated with instrument calibration are generally caused by the nonavailability or lack of primary PCBs standards, and the possibility of using secondary standards such as well-defined commercial PCBs mixtures. The application of these newer methods to environmental, biological, and process stream samples are discussed.

The discussion summary is a review of the advantages and limitations of the various methodologies. Topics include procedures for analysis of PCBs contaminants, validation methodology, limits of detection, PCBs pattern recognition, and the cost/benefit ratio of packed versus capillary column gas chromatography.

RECENT ADVANCES IN THE ANALYSIS OF POLYCHLORINATED BIPHENYLS IN ENVIRONMENTAL AND BIOLOGICAL MEDIA

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INTRODUCTION

Polychlorinated biphenyls (PCBs), terphenyls (PCTs) and quadphenyls (PCQs) had been manufactured as commercial mixtures [Aroclors® (US), Clophen® (Germany), and Kanoclor® (Japan)] for more than four decades before they were banned in the US in 1976. Because of their long-term heavy usage as dielectric fluids, in transformers and capacitors, in hydraulic fluids, fire retardants, etc., and their persistence, PCBs have permeated into practically every environmental medium throughout the world (Hutzinger et al. 1974). Because of their ubiquity PCBs have received considerable attention in recent years in the area of analytical measurements and toxicology.

Since environmental pollution by PCBs first became apparent, a plethora of purification procedures, gas chromatographic systems, detectors and methods for quantifying gas chromatographic responses has been reported to determine the contamination of environmental and biological samples (Hutzinger et al. 1974, Sherma 1975, Chau and Sampson 1975, Leo et al. 1976, Krull 1977, Brinkman et al. 1978, Pomerantz et al. 1978, Stalling et al. 1979a,b, Sherma

1981, Albro et al. 1979). However, until recent advances in analytical instrumentation, most of the conventional methodology yielded only semi-quantitative data with virtually no qualitative or quantitative information on the isomer composition for PCBs in the sample. The analytical problems are complicated by the fact that there are 209 individual chlorinated biphenyl isomers (Ballschmitter and Zell 1980) spanning 10 homologous series (1 to 10 chlorines per biphenyl). A commercial mixture (e.g., Aroclor) itself may contain as many as 60 chlorobiphenyl isomers representing several homologs (Ballschmitter and Zell 1980).

The conventional quantification method is based upon packed column gas chromatography (GC) and reports the PCB content in environmental and biological samples by referring it to a particular Aroclor mixture (e.g., Aroclor 1242, 1254 or 1260). The GC detector is first calibrated using commercial Aroclor mixtures, and then the appropriate commercial Aroclor profile is matched to the sample profile. Using the Webb-McCall technique or a variation of it the total PCB content is calculated (Webb and McCall 1973). This approach, however, is potentially subject to considerable error.

Environmental contamination may be derived from Aroclor mixtures or from incidentally generated chlorobiphenyls whose profiles do not resemble Aroclor patterns (Pittaway et al. 1981). In either case, the conventional quantification method is inadequate. As time passes, the "Aroclor patterns" undergo alteration in the environment since selective weathering and biotransformation and bioaccumulation in living organisms perturb these patterns. The problem of Aroclor pattern dissimilarity will be further aggravated as PCB monitoring continues into the future.

The quantification problem is further exacerbated by production of chlorobiphenyl isomers in chemical process streams, incineration, etc., via chemical or pyrolysis reactions which are not the same as the chemical reactions once used for the manufacture of Aroclors (Pittaway et al. 1981). There is no reason to believe that incidental generation produces any fixed pattern of chlorobiphenyl isomers which the analyst can use to identify and quantify chlorobiphenyls based upon pattern recognition from the packed column gas chromatogram. Thus, the qualitative and quantitative analysis of PCBs in environmental and biological samples and in samples from process streams involves the difficult issue of having to detect, identify and quantify each individual isomer.

The accuracy of PCB determination in environmental, biological and process stream samples is, in addition to the reasons given above, also related to the degree of variability in the analytical response of each chlorobiphenyl isomer to the detector employed (Hutzinger et al. 1974, Boe and Egaas 1979, Albro et al. 1981, Safe et al. 1975).

Recent theoretical and experimental studies have indicated that the biological properties of chlorobiphenyl isomers are significantly influenced by the number and biphenyl ring position of the chlorine atoms (McKinney and Singh 1981, Poland and Glover 1977, Matthews et al. 1978, Kimbrough 1980). Because the toxicological properties vary considerably among isomers (McKinney and Singh 1981, Poland and Glover 1977), more sophisticated methods capable of yielding information about the chemical composition at the isomeric level are

required in order to precisely investigate and assess the toxicological consequences of PCB pollution. Obviously, the ideal analytical procedure is one which identifies and measures each individual chlorobiphenyl isomer.

This chapter examines the most recent advances which strive to meet the objective of individual chlorobiphenyl isomer identification and quantification. The areas discussed are: (a) high resolution gas chromatography; (b) detection systems; (c) availability of chlorobiphenyl isomer standards; and (d) application of state-of-the-art methods to the analysis of environmental and biological samples. It is beyond the scope of this paper to include sampling techniques and isolation and purification methods. These have been adequately reviewed elsewhere.

HIGH RESOLUTION GAS CHROMATOGRAPHIC TECHNOLOGY

In order to analyze for 209 individual chlorobiphenyl isomers the use of high resolution chromatographic techniques is mandatory. In this respect, GC currently is far superior to high resolution thin-layer and high resolution liquid chromatography for PCB analysis. The relative merits of packed column (low resolution) versus capillary column (high resolution) GC analysis of PCBs have been succinctly reported by Mullin and Filkins (1981). Their work provided the impetus for further research into the determination of an optimum capillary column(s) for the analysis of individual isomers of PCBs, polybrominated biphenyls, pesticides and other halogenated hydrocarbons in biological and environmental samples.

Development of Capillary Columns

Moseley and Pellizzari have recently reported on extensive investigation into five variables which affect capillary performance for the analysis of PCBs (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). These variables are: (a) material of construction; (b) pretreatment/deactivation procedures; (c) stationary phase type; (d) stationary phase film thickness; and (e) capillary dimensions. Evaluation criteria employed were: (a) separation number (T2) between 2,2',4',5-tetrachlorobiphenyl and 2,2',4,4',6,6'-hexachlorobiphenyl; (b) resolution between 2,2',5,5'-tetrachlorobiphenyl and 2,2',4',5-tetrachlorobiphenyl; (c) height equivalent to an effective theoretical plate (HETP) for 2,2',4',5-tetrachlorobiphenyl; (d) adsorption characteristics; (e) thermal stability; and (f) general performance on an Aroclor 1242/1260 mixture (1:1, w/w).

Capillaries were made from pyrex and soft glass, quartz, vitreous silica and fused silica materials (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). The objective of this facet of the study was to determine the most suitable material for construction, pretreatment/deactivation, and amenability to coating of a thin, uniform, stable film of stationary phase. It was recognised early in this work that these variables were not independent of each other. Except for C-87, all stationary phases evaluated could be coated on the flexible silica capillaries (Pellizzari et al. 1981). Preference for the silica capillaries was attributed to their flexible nature facilitating their assembly into GCs even by the novice. Thus, a major impediment to the use of capillaries by the analytical community had been removed.

Many pretreatment/deactivation procedures were investigated since the raw construction material was not suitable for directly coating with stationary phase (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). The procedures were: (a) barium carbonate treatment; (b) Carbowax 20M; (c) Superox-4; (d) HCl etching; (e) persilylation; and (f) thermally induced polysiloxane (SE-52 or OV-101) bonding. The preferred methods were polysiloxane deactivation on silica and pyrex and persilylation on pyrex (Moseley and Pellizzari 1982b, Pellizzari et al. 1981).

Although glass capillaries coated with C-87 stationary phase provide excellent resolution of individual chlorobiphenyl isomers, use of C-87 currently is limited by two factors - its temperature stability (~220°C) and the current inability to successfully coat a uniform film on silica. Because of the upper temperature limit the C-87 phase coated capillaries are inadequate for the analysis of PCTs, PCQs, PBBs and sample extracts which are very "dirty" (e.g., fish). For these reasons alternate stationary phases were sought.

The current literature reports several different phases used in capillary GC for analysis of PCBs (Albro et al. 1981, Mullin and Filkins 1981, Krupcik et al. 1976, Krupcik et al. 1980, Albro et al. 1977, Stalling et al. 1978, Trunstra et al. 1981); however, until our research there was not a concerted effort to systematically evaluate phases (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). Using the previously mentioned criteria a matrix designed study was executed in concert and while making a comparison of phase selectivity (McReynolds' constants) to guide the overall investigation toward the "optimum" phase. Among the phases evaluated were C-87, SE-54, SP-2100, QF-1, SE-52, OV-101, Dexsil 410, Apiezon M and Apiezon L. Early results predicted (from McReynolds' constants) that Apiezon M would most closely mimic the excellent separation pattern of C-87 (Pellizzari et al. 1981). In fact, this correspondence was experimentally demonstrated (Moseley and Pellizzari 1982a). Several advantages of Apiezon M were recognized and will be discussed later. Finally, a complementary stationary phase to Apiezon M was sought so that one phase could serve as a primary analytical column and the other a reference column. SE-54 coated capillaries provided a significantly different resolution pattern compared to Apiezon M and SE-54 was also selected in our research (Moseley and Pellizzari 1982a,b).

It has long been recognized that the thinner the stationary film thickness the higher the mass transfer coefficient. The sample capacity, however, decreases. Thus, research was performed to determine a film thickness which had a very high mass transfer coefficient (as measured by HETP), adequate sample capacity for the detection system to be employed, and stability to long periods of usage (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). Stability (to solvent and thermal shock) could, of course, be imparted by immobilizing the film on the silica surface by a crosslinking/surface bonding reaction (Moseley and Pellizzari 1982b).

Two methods were reported which immobilized phases to silica (Moseley and Pellizzari 1982b). One utilized a thermal technique (SE-52), the second a dicumyl peroxide reaction (SE-54). The first method easily produced the desired 0.025 µ film capillaries for use with electron capture (EC) and negative ion chemical ionization (NICI) mass spectrometric (MS) detection of

PCBs. These capillaries exhibited a bleed of 0.7 pA (flame ionization detection) at 320°C. The second method yielded thicker films (0.1 μ) for higher capacity for use with electron impact MS.

An Apiezon M phase immobilized to silica has not been reported.

The results of these investigations which specifically focused on resolution of PCBs indicated that a silica capillary, polysiloxane deactivated, 0.2-mm ID x 50-m in length, coated with Apeizon M (0.025 μ film) or SE-54 (0.025-μ film) is preferred (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981). An Apiezon M coated silica capillary allows the elution of PCBs to occur approximately 30-40°C lower than other stationary phases (Moseley and Pellizzari 1982a,b, Pellizzari et al. 1981) and is thermally stable to ~285°C.

Comparison of Packed and Capillary Column Profiles

The inherent differences between low and high resolution gas chromatographic columns are exemplified by Figures 1 and 2. Figure 1 compares the profiles for a standard mixture of Aroclor 1242/1260. Figure 2 depicts the profiles for a stack (stationary source) sample (Mullin 1981). Both high resolution analyses were performed on SE-54 fused silica capillaries.

It is evident in Figure 2 that the low resolution profile does not readily resemble a commercial Aroclor mixture. Thus, it would be highly inaccurate to quantify the low resolution profile using a commercial Aroclor standard for instrument calibration and the Webb-McCall method (Webb and McCall 1973).

Several examples demonstrating the performance of Apiezon M silica capillaries will be discussed later in this chapter.

DETECTION SYSTEMS

Modes of Detection

Use of several types of detectors has been reported over the years in the analysis of PCBs. However, only a few have made significant recent advances that are noteworthy. These are electron capture, negative ion chemical ionization mass spectrometry (NICI-MS) and selected ion monitoring (SIM), a variant of electron impact mass spectrometry.

Electron Capture Detection

The electron capture detector continues to be one of the most sensitive and hence valuable selective detectors for PCB detection. It is only recently, however, that its full potential has been realized and incorporated into commercial systems.

From a quantitative standpoint, one of the limitations of the electron capture detector in past has been nonlinearity of response. Until the mid-1970s the ECD in use had a linear dynamic range of approximately 50-100. Several papers have dealt with determining the proper function which would give a linear relationship with concentration (Pellizzari 1974, Wentworth and

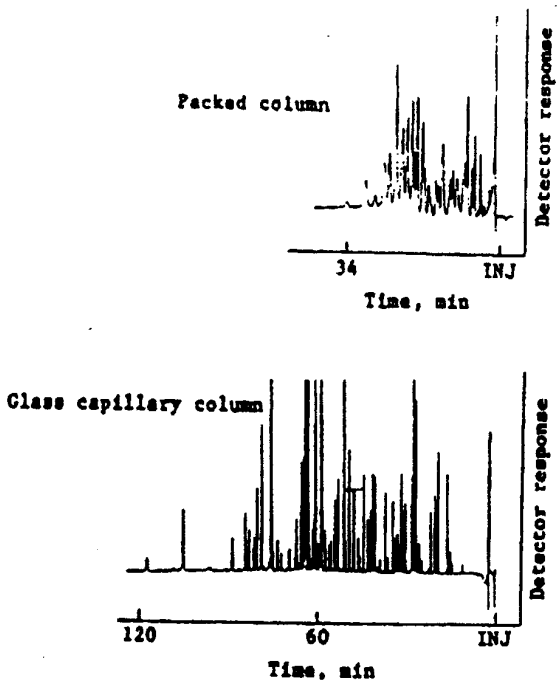

Figure 1. Chromatograms of Aroclor 1242/1260.
Top - packed column, bottom - capillary.

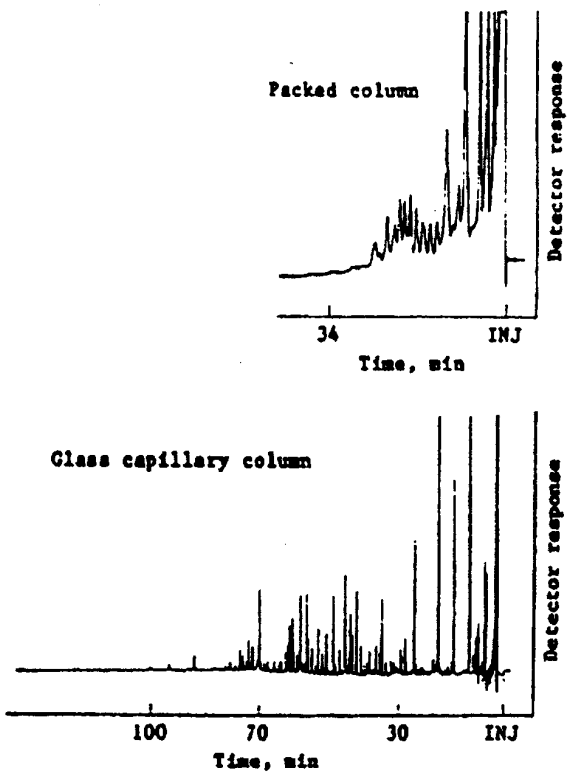

Figure 2. Chromatograms of stack sample.
Top - packed column, bottom - capillary.

Chen 1979). It has been suggested that the response was logarithmic by analogy to light absorption (Wentworth and Chen 1979). However, when the ECD was operated in the pulse sampling mode the reactions occurred primarily in the field free period, so that the analogy was not considered very appropriate (Wentworth and Chen 1979).

Once a valid kinetic model for the electron capture processes for thermal reactions was derived by Wentworth et al. (1979) and the research in atmospheric pressure ionization mass spectrometry gave direct evidence on negative and positive ion formation (under ECD conditions), the solution to electron capture detector nonlinearity was in hand. Attention was focused on the ECD's concentration dependance which has been of great concern to analytical chemistry. Analysis of the kinetic model was carried out by numerical solution of differential equations which alleviated some of the early mathematical assumptions.

As a result of the experimental work on electron capture mechanisms, subsequent reports appeared in the literature describing an alternate method of linearization of response (Maggs et al. 1971, Patterson et al. 1976). The fundamental principle was based upon modulating the frequency of pulsing the detector electrodes so that the plasma current was maintained constant. This function is given by

$$K_s = \frac{F - F_0}{F_0}$$

where F_0 = the frequency giving the base current in the absence of capturing species

F = the frequency giving the base current in the presence of a capturing species

Initially there were reports of a "break" in linearity, but with improved instrument design parameters this problem was eliminated (Patterson et al. 1976). Thus, the modern ECD utilizes modulated pulsed frequency to achieve a dynamic range of approximately four orders of magnitude, an improvement which is necessary for the analysis of PCBs in environmental and biological samples.

A second limitation was the ECD cell volume. This problem did not surface until analysts began investigating the use of high resolution capillary columns (Pellizzari 1974). The original cell volumes of 2-4-mL were adequate when used with packed columns whose flows were high and chromatographic peak shape and efficiency were preserved. A few isolated reports appeared in the literature addressing improved cell design, in particular low volume cells, for use with capillaries. ECD cell volumes between 250 and 500-µL utilizing a coaxial design still required a scavenger gas after the capillary column to reduce the residence time in the ECD cell and preserve the ultra-high theoretical plates which were attainable by capillaries (Pellizzari 1974). However, it was only recently that commercial GC systems became routinely available with ECDs compatible with capillery flow rates.

With these two parallel developments it is now feasible to perform elegant quantitative electron capture detection of PCBs.

Negative Ion Chemical Ionization Mass Spectrometry

This detection method is a variant of positive ion chemical ionization mass spectrometry. The associated electronics to regulate, focus and pass negative ions to an appropriately polarized electron multiplier were developed in the mid-1970s; however, it was not until recently that ion source and reagent gas conditions were investigated for optimizing PCB analysis (Pellizzari et al. 1981).

NICI-MS is uniquely suited to measuring trace quantities of polyhalogenated chemicals in environmental samples because of its high sensitivity for these chemicals and its virtual transparency to otherwise potentially interfering molecules (Kuehl et al. 1980). It also provides, in addition to sensitivity, molecular ion information and thus a verification of the structural entity being measured, a highly desirable feature when examining complex environmental and biological samples.

NICI-MS is very closely analogous to electron capture detection in that the ion forming reactions are common to both. The ion forming reactions that are important for polychlorinated molecules have been described (Kuehl et al. 1980). They include: (a) the resident capture of thermal electrons; (b) chloride attachment; (c) deprotonation; and (d) oxygen exchange. Because NICI-MS is a novel technique, the operating parameters which optimize the technique preferentially to one of the above mechanisms for PCB analysis have recently been studied in our laboratory.

Investigations in this laboratory have centered on the use of high resolution gas chromatography in combination with NICI-MS while elucidating and characterizing instrumental parameters suitable for PCB analyses (Pellizzari et al. 1981). These investigations have included: (a) examining the performance of two different ion source designs; (b) the effect of source pressure on sensitivity and spectral signature; and (c) the effect of various reagent gases on sensitivity and fragmentation of PCB isomers. Compared to conventional electron impact MS, less information about the structure of the compound is obtained. Thus, the specific aim of the investigations was to study several moderating and reagent gases to enhance: (1) the formation of molecular anions of the individual chlorobiphenyl isomers; or (2) their dissociation to yield chloride 35 and 37 isotopic anions (Pellizzari et al. 1981).

Reagent gas studies were conducted with methane, oxygen/nitrogen, nitrous oxide/nitrogen, nitrous oxide/methane, difluorodichloromethane, and tetrafluoromethane. This variety of reagent gases was necessary because a major problem encountered in NICI-MS analysis of PCBs had been the lack of molecular weight information obtained for the lower molecular weight PCBs (C_1-C_6) under methane moderated electron capture conditions. Since the lower molecular weight PCBs undergo dissociative electron capture to form Cl^- ions under these conditions, use of a reagent gas which chemically reacts with individual chlorobiphenyl isomers rather than just moderating electron energy led to the observation of useful molecular weight information in the spectral signature.

Field et al., (Smit and Field 1980) had observed that a mixture of nitrous oxide and methane produced abundant OH^- ions under negative ion

conditions. The hydroxide ions were observed to react with a wide variety of compounds by proton abstraction to form $(M-H)^-$ ions. Therefore, this reagent gas mixture was studied in our laboratory as a likely candidate to provide molecular weight information for individual chlorobiphenyl isomers.

Experiments were conducted with an LKB 2091 magnetic sector instrument with a relatively open source design. Figures 3-7 depict mass spectra of monochlorobiphenyl, trichlorobiphenyl, hexachlorobiphenyl, octachlorobiphenyl, and decachlorobiphenyl obtained under N_2O/CH_4 NICI conditions. Nitrous oxide was introduced through a reagent gas inlet (5×10^{-5} Torr as measured at the Penning gauge) and methane (3×10^{-5} Torr) was passed into the ion source via a GC make-up line and separator. Based on calculations made for positive ion methane CI the actual source pressure exerted by both reagent gases was 0.2-0.3 Torr. The MS system was optimized for m/z 17 (OH^-). A 2-uL injection (~100-pg) of a standard solution of PCBs was made with a 5:1 split ratio. As indicated in Figure 3 the spectrum of 2-chlorobiphenyl exhibited $(M-H)^-$ ions. The M^- peak at m/z 188 is no larger than expected from ^{13}C isotope abundance. In addition, very low intensity ions were found for the $(M-H+O)^-$ ion at m/z 203, $(M-H+N_2O)^-$ ion at m/z 231, and $(M-H+NO)^-$ ion at m/z 217.

The high mass region for trichlorobiphenyl (> m/z 40, Figure 4) mass spectrum was dominated by the $(M-H)^-$ ions at m/z 255, 257, and 259. The M^- intensity was greater than predicted on the basis of ^{13}C isotope abundance (24% vs. 13%) suggesting that some stabilization of M^- as occurring. Also, $(M-Cl)^-$ ions were evident at m/z 221 and 223.

For hexachlorobiphenyl (Figure 5) the $(M-H)^-$ ions at m/z 357, 359, 361, and 363 were present. Also, substantial abundance of M^- ions was observed (53% observed vs. 13% calculated as ^{13}C). Ion clusters for the loss of one chlorine (m/z 323) and two chlorines (m/z 288) from the parent molecule were detected. An interesting ion cluster formed by the loss of chlorine and the addition of oxygen $(M-19)^-$ was observed (m/z 338).

The mass spectrum of octachlorobiphenyl (Figure 6) exhibited negligible hydrogen abstraction but was certainly dominated by M^- ions. Since there are only two hydrogens available for abstraction on this PCB homolog, this result was unexpected.

Decachlorobiphenyl (Figure 7) with no hydrogens available for abstraction yields a spectrum that is due to electron capture (formation of M^-) and Cl^-.

The above observations for N_2O/CH_4 reagent gas indicate that verification of the molecular weight of the chlorobiphenyl isomer can be achieved in high resolution GC NICI-MS. Furthermore, the homogeneity of gas chromatographic peaks in complex mixture analysis can be established since non-PCB substances or nonhomologous PCB isomers can be distinguished. NICI-MS will thus provide molecular weight information, which has been one of the strong suits claimed for EI-MS.

Another moderating gas typically used in NICI-MS is methane. Experiments in our laboratories on the effects of source pressure on sensitivity indicated that as the methane pressure increased so also did sensitivity. As a compromise between maximum sensitivity and excessive pressure, a reagent gas pres-

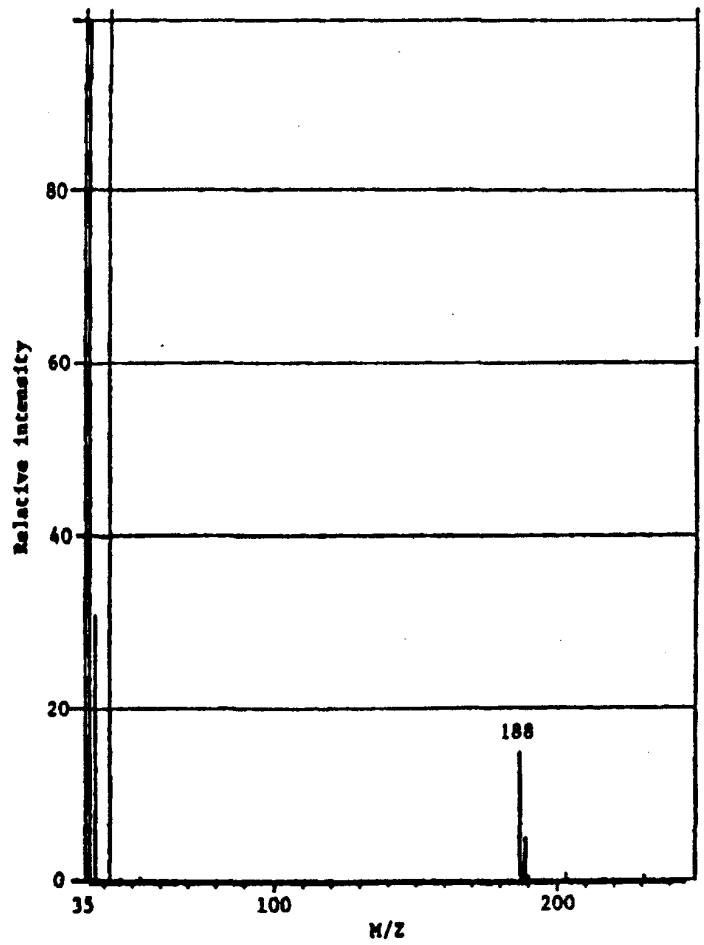

Figure 3. N_2O/CH_4 NICI mass spectrum of monochlorobiphenyl.

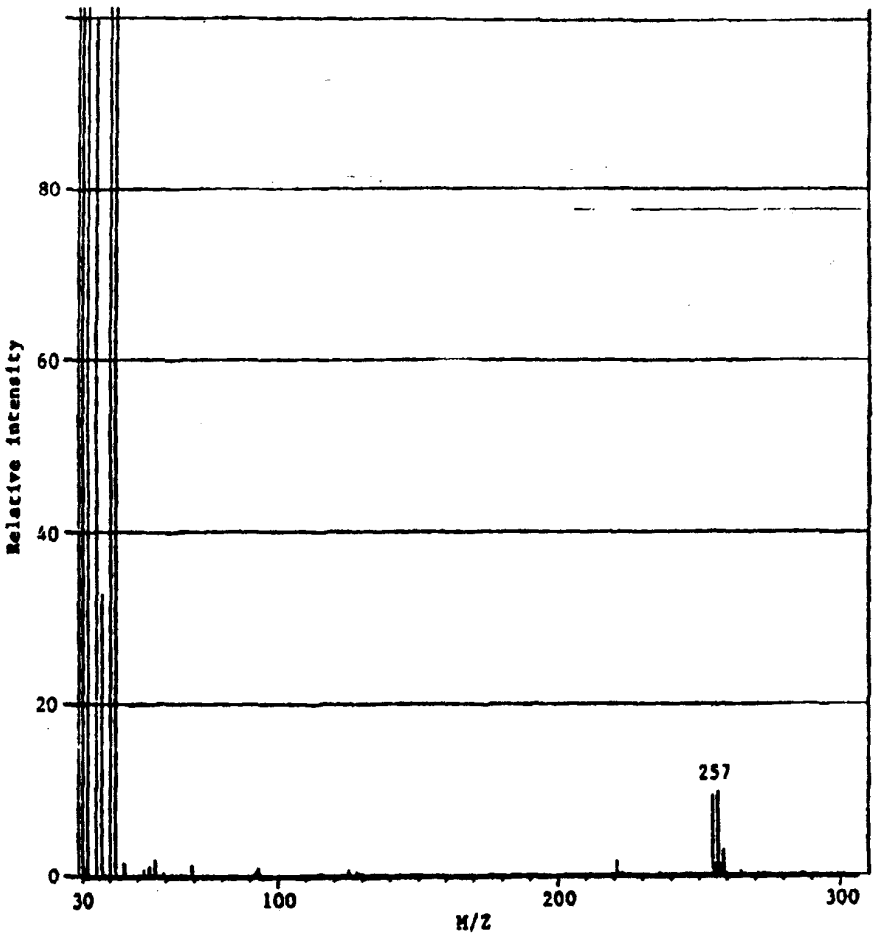

Figure 4. N_2O/CH_4 NICI mass spectrum of trichlorobiphenyl.

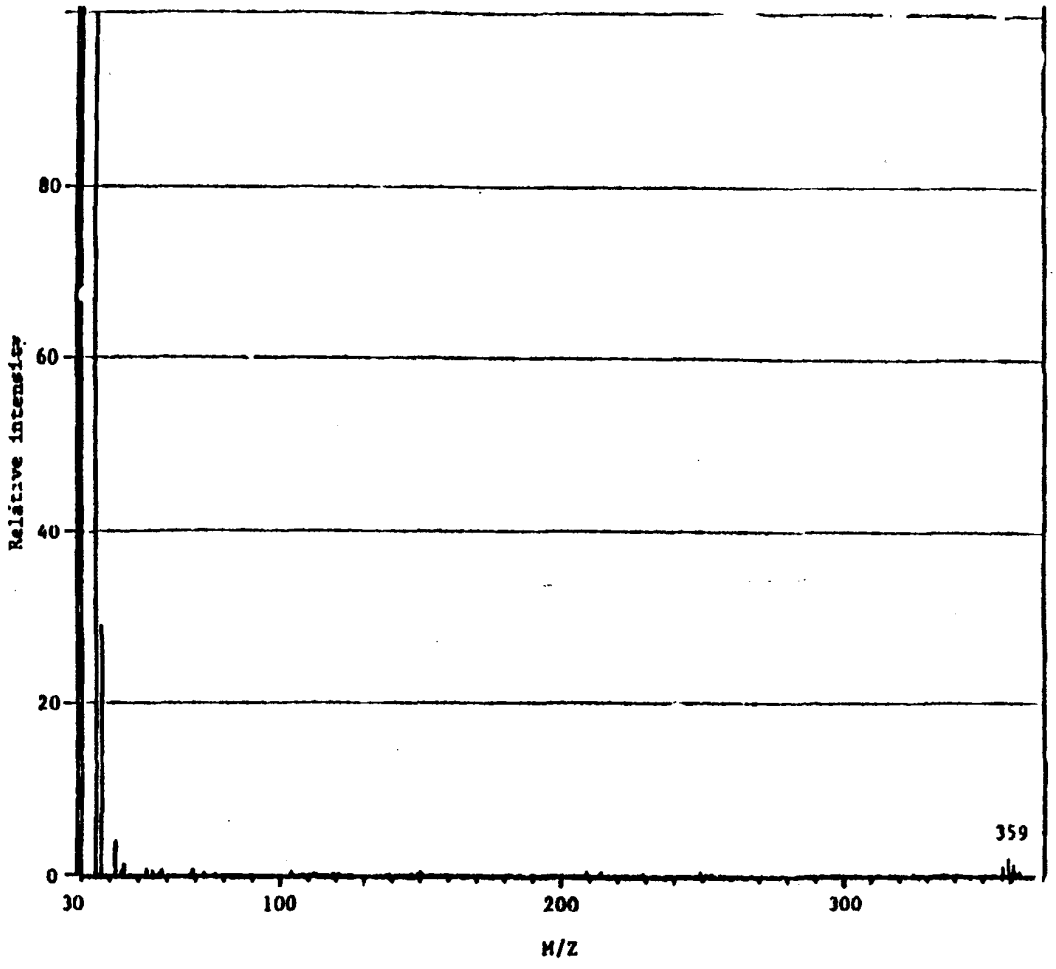

Figure 5. N_2O/CH_4 NICI mass spectrum of hexachlorobiphenyl.

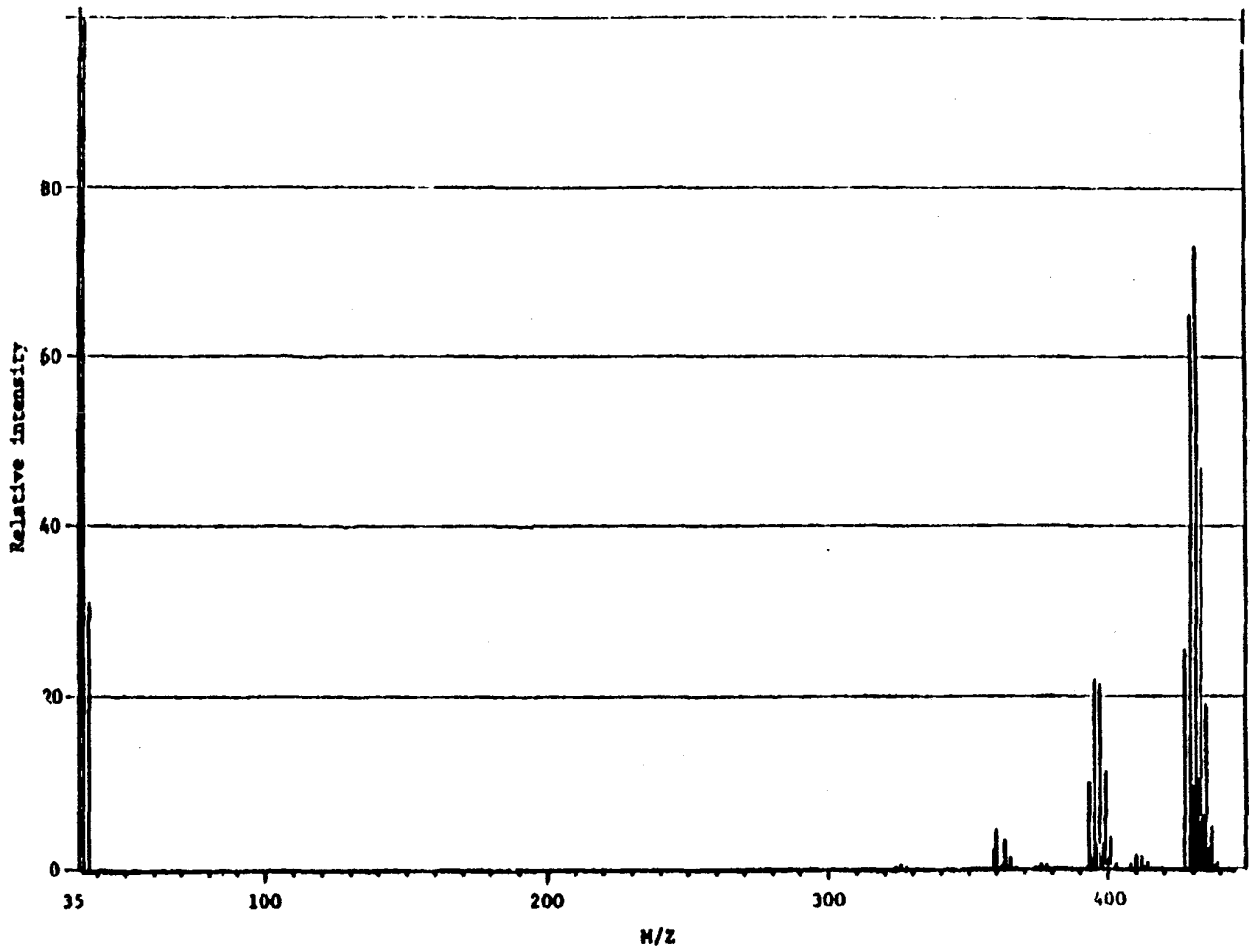

Figure 6. N_2O/CH_4 NICI mass spectrum of octachlorobiphenyl.

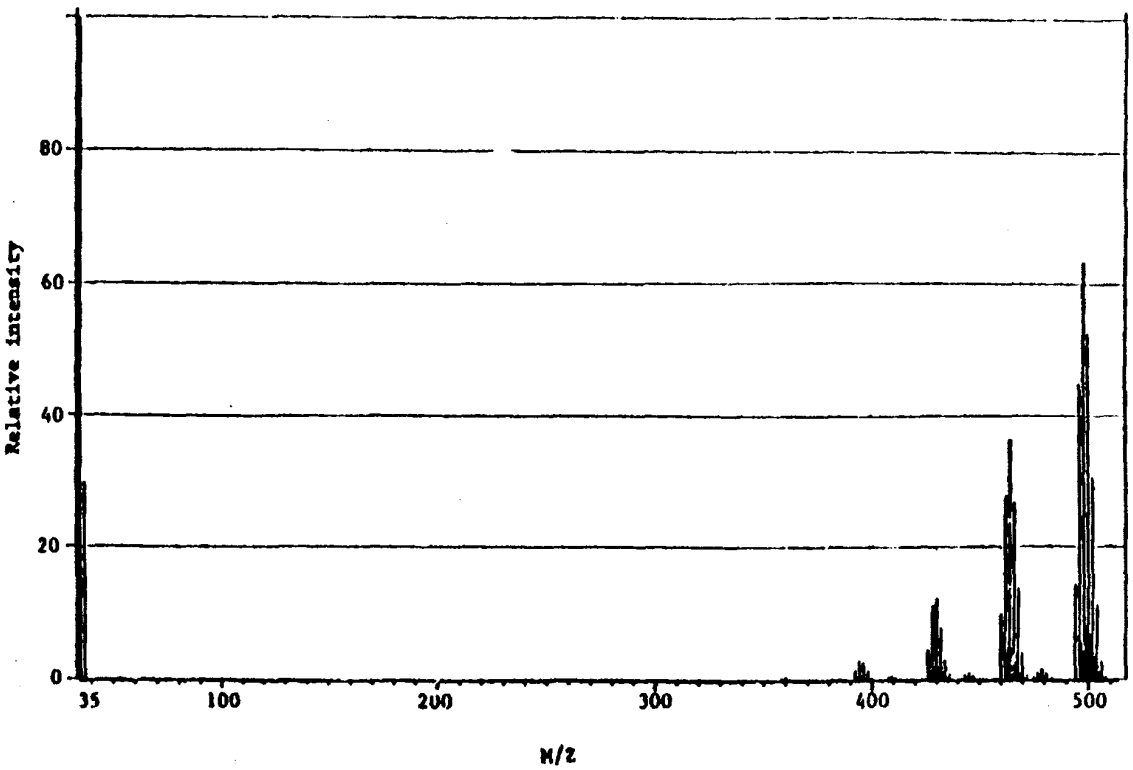

Figure 7. N_2O/CH_4 NICI mass spectrum of decachlorobiphenyl.

sure of 4×10^{-5} Torr is normally employed (measured at diffusion pump throat, actual source pressure may be higher).

Of the reagent and moderating gases studied, methane provides one of the more sensitive modes of operation. However, the principal mechanism of reaction is dissociative electron capture leading to m/z 35 and 37 which can be used for quantification.

The chemical thermodynamics in NICI-MS is also effected by the physical design of the GC/MS source where the CI takes place. The experiments conducted with a relatively open and closed source designs gave parallel results. The principal feature of the open source was that it was less subject to filament carbonization and its sensitivity was maintained stable for a few weeks. Considereble absolute differences in the limits of detection were observed between two different instrument makes. One system was clearly capable of detecting in the high femtogram region, while the other required two orders of magnitude more PCB material.

Thus, the advantages of both sensitivity and molecular weight information can be realized with NICI-MS. The recent studies have provided a basis for choosing the appropriate operating parameters for analysis of PCBs.

Selected Ion Monitoring Utilizing EI-MS

Electron impact mass spectrometry has been a very popular analytical tool, in addition to ECD, as a GC detector for PCBs. The use of the conventional scanning mode suffers from an inadequate limit of detection for chlorobiphenyl isomers. The limits of detection may differ between EI-MS and ECD by as much as three orders of magnitude (Pellizzari 1981). For this reason, EI-MS per se has not been widely successful unless there are large quantities of PCBs present in environmental and biological samples.

There have been reports, however, of a specialised application of EI-MS. Selected ion monitoring (SIM) had been primarily developed for drug analysis, but recently it has been applied to verifying and quantifying PCBs (Tindall and Wininger 1980, Martelli et al. 1981, Albro and Parker 1980, Erickson and Pellizzari 1979). Improved limits of detection are realized.

Another variant of EI-MS is limited mass scanning (LMS). The use of SIM (programmed mode) and LMS permits the spectrometer to spend more time transmitting through the analyzer to the electron multiplier ions of interest to yield lower limits of detection (Tindall and Wininger 1980). Both of these techniques are under computer control and are also available to the analyst when operating in the NICI-MS mode.

Pulsed Positive Ion Negative Ion Chemical Ionization (PPINICI) Mass Spectrometry

During the past few years PPINICI has been developed: rapid switching between positive and negative CI (12kHz) allows simultaneous information to be acquired (Pellizzari and Moseley 1981). Little research has been performed on its optimization and application to PCB analysis.

Combination of High Resolution Gas Chromatography and Ultrasensitive Detection

Figures 1 and 2 depict the combination of state-of-the-art high resolution chromatography and electron capture detection. It is clearly evident from these profiles that modern EC detectors are designed to preserve the high resolution which capillary columns are now capable of delivering.

The direct coupling of high resolution GC capillaries with the ion source of mass spectrometers has also been successfully accomplished. The flexible nature of silica capillaries has greatly facilitated this accomplishment, since most MS systems are not well engineered to accept the rigid glass capillaries.

Figure 8 depicts an example of analysis of an Aroclor 1016/1254/1260 mixture (2.5:2.0:1.0 w/w/w) utilizing an Apiezon M phase coated (0.025-μ film), FSD silica capillary and NICI-MS detection. Table 1 lists the operating conditions which were optimized for dissociative electron capture (i.e., enhancement of m/z 35 and 37). The mass chromatogram (m/z 35) exemplifies the close similarity between the proportional response for individual chlorobiphenyl isomers under methane NICI conditions and those obtained with ECD. An expanded version of this chromatogram is given in Figures 9-11. Chromatographic peaks 3, 35, and 103 are the internal standards, 1,2-dichloronaphthalene, 1,2,3,4-tetrachloronaphthalene, and octachloronaphthalene, respectively. The chromatographic peaks depicted in Figures 9-11 indicate ideal symmetry and thus the chromatography was preserved. The peak residence times are generally 7-8s. Because the ECD and NICI-MS profiles are superimposable, the numbering scheme shown in Figures 9-11 was standardized for our characterization research and for our cross-referencing and verification efforts. The identity of several of the peaks will be discussed below.

Another means of representing NICI-MS information is depicted by Figures 12 and 13. The top tracing is the one shown in Figure 8 but it is considerably reduced. The remaining profiles in Figure 12 are mass chromatograms for m/z 188 (Cl_1), 222 (Cl_2), 256 (Cl_3), 292 (Cl_4), and 326 (Cl_5) representing the five homologous series. It is important to note that under methane NICI conditions, the intensity of molecular anions for Cl_1-Cl_5 is very weak to nonexistent. It is more appropriate to use N_2O/CH_4 for detecting which chromatographic peak belongs to a homolog. Figure 13 shows mass chromatograms for m/z 360 (Cl_6), 394 (Cl_7), 428 (Cl_8), 462 (Cl_9), and 496 (Cl_{10} not detected) representing the remaining homologous series. In this case all of the chlorobiphenyl isomers were detected in the Aroclor mixture.

Variation of Detector Responses

A common problem with ECD, NICI-MS and EI-MS (SIM, LMS) as detectors is the large variation which is observed between the individual chlorobiphenyl isomers, both within and between homologous series (Hutzinger et al. 1974, Boe and Egaas 1979, Albro et al. 1981, Safe et al. 1975). The relative response factors (RRF_i) for a few individual chlorobiphenyl isomers obtained with high resolution GC-ECD [$(GC)^2$-ECD] have been reported. A majority of the isomers have been analyzed in our laboratory and the RRF_i compared (Table 2). The RRF_i was calculated as follows:

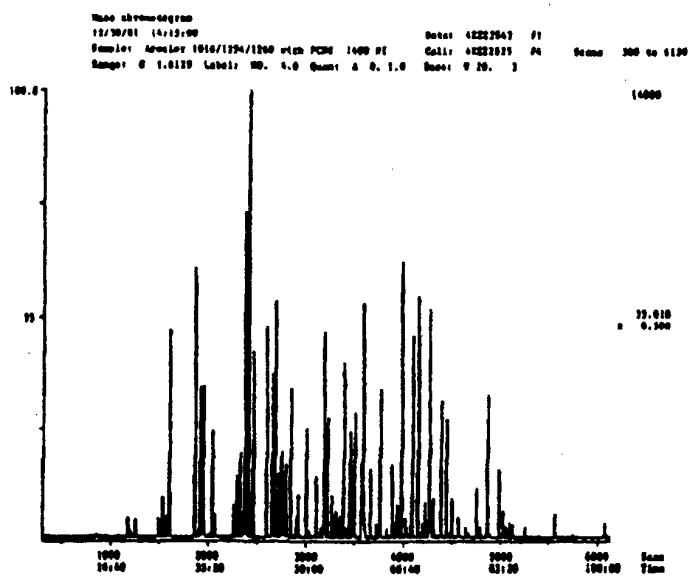

Figure 6. Mass chromatogram (m/z 35) of Aroclor 1016/1254/1260 obtained by CH_4 NICI-MS.

Table 1. $(GC)^2$/NICI-MS Operating Conditions (Finnigan 4021)

Parameter	Condition
GC	
Capillary	Fused silica
ID	0.23 mm
Length	45 m
Deactivation	Polysiloxane
Stationary phase	Apiezon H
Film thickness	0.025 μ
He carrier	0.6 mL/min (32 cm/sec)
Splitless/split	40 sec; 10:1
Temperature	100°C/0.1 min → 260°C @1.5°C/min
MS	
Reagent gas	CH_4
Forepressure	0.2 torr
High vacuum pressure	4.2×10^{-5} torr
Manifold temperature	120°C
Ionizer temperature	260°C
Emission current	0.5 mA
Electron energy	70 eV
Scan cycle	1.0 sec
Scan range	30-700 daltons

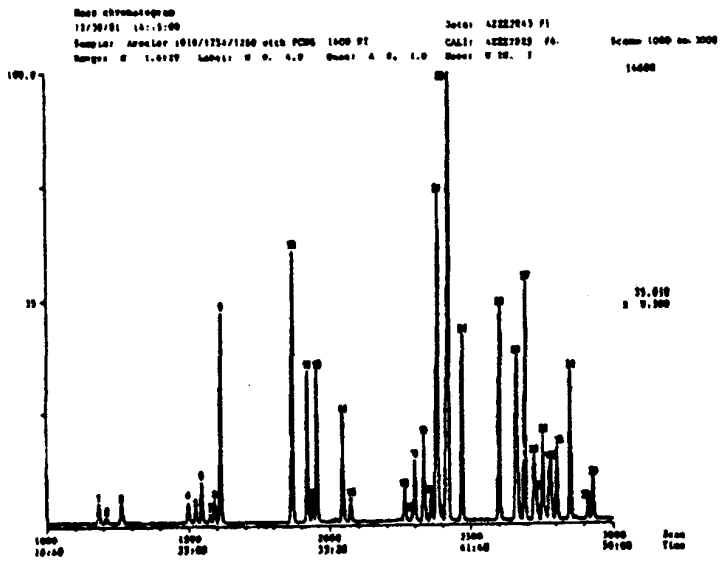

Figure 9. Mass chromatogram (m/z 35) of Aroclor 1016/1254/1260 obtained by CH_4 NICI-MS (Figure 8 expanded).

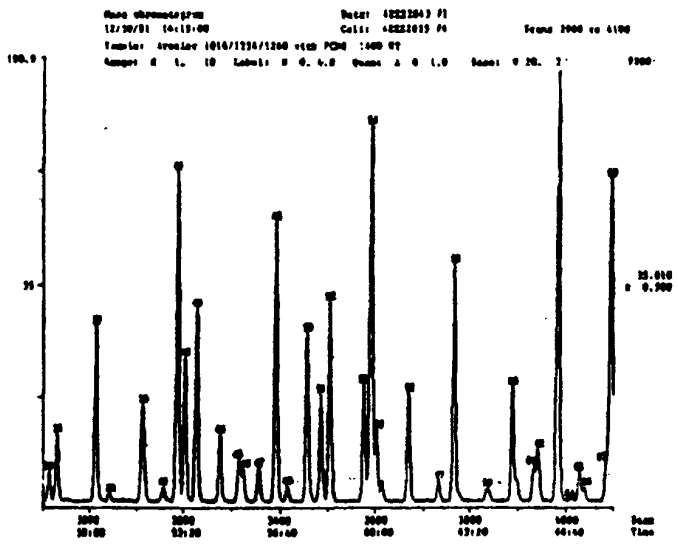

Figure 10. Mass chromatogram (m/z 35) of Aroclor 1016/1254/1260 obtained by CH_4 NICI-MS (Figure 8 expanded--middle portion).

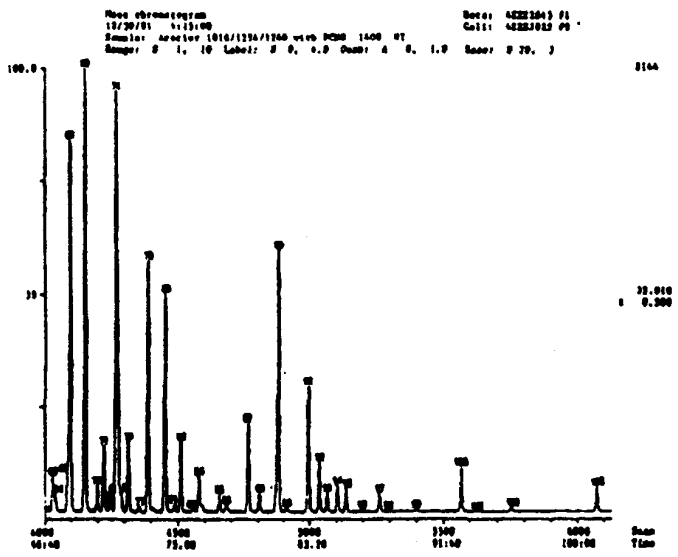

Figure 11. Mass chromatogram (m/z 35) of Aroclor 1016/1254/1260 obtained by CH_4 NICI-MS (Figure 8 expanded--later portion).

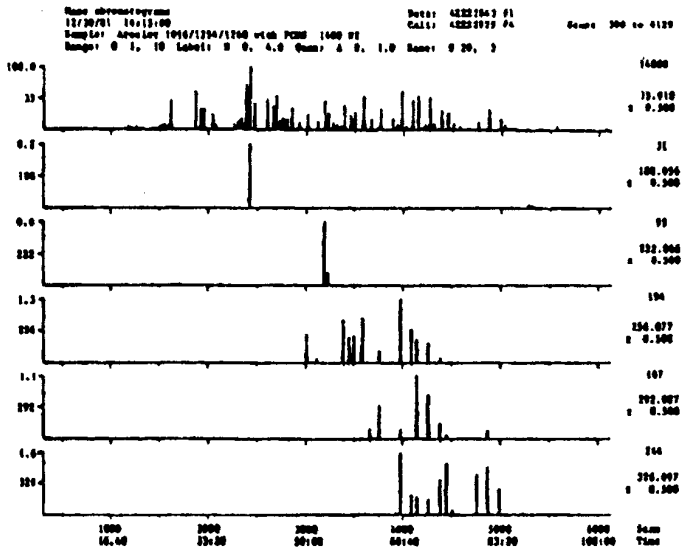

Figure 12. Mass chromatogram for parent ions of each PCB homolog (Cl_1-Cl_5) in Aroclor 1016/1254/1260 mixture.

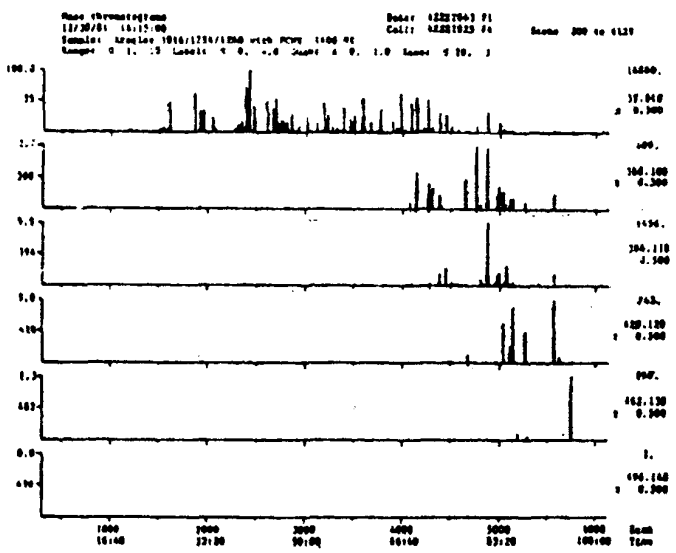

Figure 13. Mass chromatogram for parent ions of each PCB homolog (Cl_6-Cl_{10}) in Aroclor 1016/1254/1260 mixture.

Table 2.– Relative Response Factors for Individual PCB Isomers Using $(GC)^2/ECD$

Homolog (No.)[a]	PCB Isomer No.[a]	RRF_i (%RSD)		Range	Mean ± S.D. (%RSD)	N[e]
1Cl(3)	1	5.041 (3.9)	[b]			
	2	11.470 (2.5)		5.041-13.143	9.885 ± 4.27 (43)	3
	3	13.143 (6.8)				
2Cl(12)	4	3.555 (4.1)	[b]			
	5	2.925 (5.9)				
	7	0.523 (5.6)				
	9	0.376 (3.4)				
	10	0.619 (1.8)		0.142-3.555	1.427 ± 1.28 (90)	9
	11	2.726 (1.2)				
	12	1.231 (1.3)				
	14	0.749 (0.8)				
	15	0.142 (3.9)				
3Cl(24)	18	0.705 (3.9)	[c]			
	21	0.313 (0.5)				
	22	0.598 (4.6)				
	26	2.035 (0.9)				
	29	1.514 (2.0)		0.313-2.035	1.137 ± 0.65 (58)	9
	30	0.321 (4.4)				
	31	1.706 (2.9)				
	33	1.389 (2.2)				
	37	1.656 (2.7)				
4Cl(42)	40	0.422 (1.2)	[c]			
	42	1.252 (5.8)				
	43	0.471 (0.6)				
	44	1.086 (2.6)				
	45	1.472 (0.7)				
	46	1.585 (0.7)				
	47	0.905 (3.3)				
	48	0.460 (0.4)				
	49	1.008 (1.6)				
	50	1.150 (1.0)				
	52	1.176 (2.5)				
	53	1.504 (1.2)				
	54	0.643 (4.4)				
	56	1.004 (0.6)				
	58	1.632 (1.2)		0.367-2.124	1.024 ± 0.39 (38)	31
	60	1.160 (3.8)				
	61	0.367 (0.3)				
	62	0.918 (1.0)				
	63	1.138 (0.6)				
	65	0.479 (5.3)				
	66	1.229 (2.3)				
	68	0.812 (1.3)				
	69	0.866 (1.5)				
	70	0.978 (8.4)				

Table 2 (continued)

Homolog (No.)[a]	PCB Isomer No.[b]	RRF_i (%RSD)	Range	Mean ± S.D. (%RSD)	N[e]
4Cl(42)	71	1.036 (0.3)			
(cont.)	72	1.039 (3.1)			
	73	0.812 (1.3)			
	75	1.096 (1.4)			
	77	2.124 (0.5)			
	78	0.831 (0.7)			
	81	1.097 (0.3)			
5Cl(46)	82	1.123 (0.6) [c]			
	83	0.440 (3.4)			
	85	1.358 (11.4)			
	86	1.094 (2.5)			
	87	0.999 (18.3)			
	88	0.697 (0.6)			
	92	0.904 (0.3)			
	93	1.226 (1.1)			
	94	1.456 (1.1)			
	96	1.092 (0.1)			
	97	1.047 (3.7)			
	98	1.231 (0.8)			
	100	0.944 (3.8)			
	101	1.058 (5.7)			
	103	0.857 (2.2)			
	104	0.986 (0.8)			
	106	1.324 (1.9)			
	108	1.242 (2.4)	0.440-8.079	1.206 ± 1.23 (102)	35
	109	0.450 (3.3)			
	110	1.461 (1.5)			
	111	1.059 (1.3)			
	112	0.984 (13.6)			
	113	0.875 (1.5)			
	114	1.382 (2.1)			
	115	0.879 (4.8)			
	116	0.641 (4.7)			
	117	0.901 (1.8)			
	118	1.288 (2.6)			
	120	1.031 (1.6)			
	121	0.388 (1.2)			
	122	1.243 (2.6)			
	123	0.709 (3.2)			
	124	0.509 (4.2)			
	126	8.079 NQ[d]			
	127	1.242 (2.4)			
6Cl(42)	128	0.952 (1.2) [c]			
	129	0.882 (7.3)			
	130	1.384 (3.8)			

Table 2 (continued)

Homolog (No.)[a]	PCB Isomer No.[b]	RRF_i (%RSD)	Range	Mean ± S.D. (%RSD)	N[e]
6Cl(42) (cont.)	131	1.484 (0.8)			
	132	0.835 (1.0)			
	133	1.090 (2.0)			
	136	1.141 (5.7)			
	137	0.381 (5.7)			
	138	0.938 (3.6)			
	139	0.374 (2.3)			
	140	1.119 (1.5)			
	141	0.789 (4.0)			
	142	0.948 (0.7)			
	143	0.632 (0.9)			
	144	0.913 (4.4)			
	145	1.163 (8.5)			
	147	1.186 (2.9)			
	148	0.962 (1.8)			
	149	1.822 (1.6)	0.374-1.822	0.927 ± 0.32 (35)	37
	150	0.878 (0.9)			
	151	0.889 (3.7)			
	152	0.529 (3.2)			
	153	0.942 (1.9)			
	154	0.951 (0.8)			
	155	1.463 (0.6)			
	156	0.926 (1.5)			
	157	1.226 (0.7)			
	158	0.508 (13.6)			
	159	1.221 (0.7)			
	160	0.653 (1.6)			
	161	0.455 (8.5)			
	163	0.786 (1.7)			
	164	0.695 (12.2)			
	165	1.003 (1.4)			
	166	0.471 (16.3)			
	167	1.097 (3.6)			
	168	0.628 (1.0)			
7Cl(24)	170	2.317 (0.9) [c]			
	171	1.189 (2.7)			
	172	1.195 (5.3)			
	173	0.894 (2.8)			
	174	1.356 (3.2)			
	176	0.383 (0.8)			
	177	1.142 (7.6)			
	178	1.169 (3.9)			
	180	1.421 (1.6)			
	181	0.679 (14.7)			
	182	1.350 NC[d]	0.383-2.317	1.161 ± 0.40 (34)	21

Table 2 (continued)

Homolog (No.)[a]	PCB Isomer No.[f]	RRF_i (%RSD)		Range	Mean ± S.D. (%RSD)	N[e]
7Cl(24) (cont.)	183	1.079 (1.4)				
	184	1.159 (4.4)				
	185	1.387 (1.2)				
	186	0.771 (6.0)				
	187	1.359 (1.1)				
	188	1.584 (2.5)				
	190	1.254 (0.6)				
	191	0.668 (9.6)				
	192	0.858 (4.4)				
	193	1.176 (1.5)				
8Cl(12)	194	1.026 (3.9)	[d]			
	195	2.602 (7.7)				
	196	1.889 (3.0)				
	197	0.959 (8.2)				
	199	0.925 (2.5)				
	200	2.470 (1.9)		0.925-2.602	1.514 ± 0.679 (45)	10
	201	2.114 (3.1)				
	202	0.976 (2.7)				
	204	1.038 (4.2)				
	205	1.139 (1.2)				
9Cl(3)	206	1.053 (1.9)	[d]			
	207	1.816 (4.3)		1.005-1.816	1.291 ± 0.45 (35)	3
	208	1.005 (1.8)				
10Cl(1)	209	1.168 (2.7)	[d]	-	-	-

[a](No.) = number of theoretical isomers possible.

[b]Internal standard = 1,2-Dichloronaphthalene.

[c]Internal standard = 1,2,3,4-Tetrachloronaphthalene.

[d]Internal standard = Octachloronaphthalene.

[e]N = number of isomers studied; each isomer was measured in triplicate.

[f]See Ballschmitter and Zell 1980.

[g]NG = not given.

$$RRF_i = \frac{\frac{Amt_i}{A_i}}{\frac{A_{i.s.}}{Amt_{i.s.}}}$$

where:

RRF_i = relative response factor for PCB isomer i
Amt_i = amount PCB isomer i injected
A_i = peak area
$A_{i.s.}$ = peak area for internal standard
$Amt_{i.s.}$ = internal standard amount

The percent relative standard deviation for the RRF_is within a homologous series ranged from 35 to 102%. Thus, for accurate quantification of individual chlorobiphenyl isomers by $(GC)^2$-ECD, the appropriate RRF must be employed. The largest variation is observed with the Cl_4 homologous series. As additional chlorine substituents are introduced onto the biphenyl nucleus, the limits of detection do not significantly decrease and the response variation decreases since ring substitution pattern no longer plays a large role in determining the magnitude of response. This is not the case, of course, with the lower homologous series where the ring substitution pattern and number of chlorine substituents are important determinants of ECD response.

In a parallel study, the relative molar response (RMR_i for m/z 35) was determined for individual chlorobiphenyl isomers using $(GC)^2$NICI-MS. The operating conditions were previously given in Table 1. The RMR was calculated as follows:

$$RMR_i = \frac{A_i}{A_{i.s.}} \times \frac{MW_i}{MW_{i.s.}} \times \frac{Amt_{i.s.}}{Amt_i}$$

where:

A_i = area of PCB isomer i
$A_{i.s.}$ = area of internal standard
MW_i = molecular weight of PCB isomer i
$MW_{i.s.}$ = molecular weight of internal standard
$Amt_{i.s.}$ = amount of internal standard
Amt_i = amount of PCB isomer i

The percent relative standard deviation for the RMR_is within a homologous series ranged from 33 to 125% (Table 3). This data base is being expanded to include all available isomers, and no conclusions should be drawn at this time. Nevertheless, it is anticipated that appropriate RMR factors must be employed for accurate quantification.

A summary comparison of response factors is given in Table 4 for ECD, NICI-MS, and EI-MS (SIM). The literature values for EI-MS unfortunately are normalized to the lowest response within a homologous series. Thus, a comparison between homologs is not possible (Martelli et al. 1981). On the other hand, it can be readily seen that EI-MS exhibits the lowest %RSD within a

Table 3. Relative Molar Responses (m/z 35) for Individual PCB Isomers Using (GC)[a]/NICI-MS

Homolog (No.)[a]	PCB Isomer No.[a]	RRF_i (%RSD)	Range	Mean ± S.D. (%RSD)	N[e]
1Cl(3)	1	0.356 (6.8) [b]			
	2	0.105 (6.2)	0.091-0.356	0.184 ± 0.15 (81)	3
	3	0.091 (5.0)			
2Cl(12)	5	0.574 (10) [b]			
	7	4.223 (11)			
	9	2.064 (0.6)			
	10	2.430 (4.4)	0.574-4.223	1.662 ± 1.23 (74)	8
	11	0.574 (4.6)			
	12	1.452 (5.0)			
	14	1.209 (1.3)			
	15	0.773 (1.0)			
3Cl(24)	18	0.340 (12) [c]			
	21	0.842 (3.1)			
	22	5.143 (2.6)			
	26	0.623 (3.1)	0.340-5.143	1.378 ± 1.72 (125)	7
	29	0.462 (4.3)			
	30	1.686 (4.4)			
	31	0.555 (1.9)			
4Cl(42)	40	1.240 (6.3) [c]			
	42	1.208 (3.0)			
	44	0.920 (4.7)			
	47	1.447 (2.5)			
	53	0.048 (16)			
	54	0.358 (0.5)			
	55	0.779 (5.3)			
	60	2.013 (1.9)	0.048-2.013	0.971 ± 0.48 (50)	6
	61	1.562 (2.3)			
	65	0.789 (0.6)			
	69	1.055 (4.6)			
	70	0.989 (8.4)			
	72	0.840 (18)			
	75	1.038 (1.7)			
	77	0.364 (3.9)			
	81	0.893 (1.7)			
5Cl(46)	85	1.077 (2.0) [d]			
	87	0.564 (8.0)			
	93	0.528 (4.6)			
	101	0.818 (8.0)			
	106	1.123 (4.8)			
	112	0.667 (3.1)	0.465-1.216	0.805 ± 0.27 (33)	12
	114	1.137 (4.0)			
	116	1.216 (1.8)			

Table 3 (continued)

Homolog (No.)[a]	PCB Isomer No.[b]	RRF_i (%RSD)		Range	Mean ± S.D. (%RSD)	N[e]
5Cl(46)	117	0.839 (2.5)				
(cont.)	118	0.465 (8.4)				
	121	0.634 (5.5)				
	122	0.591 (2.3)				
6Cl(42)	128	1.011 (4.8)	[d]			
	129	0.901 (8.4)				
	131	1.256 (4.8)				
	133	0.559 (2.7)				
	136	0.396 (4.9)				
	137	0.911 (4.6)				
	139	0.747 (4.1)				
	141	1.440 (8.6)		0.369-1.440	0.817 ± 0.29 (36)	16
	147	1.042 (6.8)				
	151	1.030 (7.3)				
	155	0.369 (2.9)				
	159	0.525 (0.7)				
	160	0.735 (2.5)				
	161	0.719 (2.3)				
	163	0.688 (4.4)				
	165	0.738 (3.9)				
7Cl(24)	170	0.451 (10)				
	173	0.659 (0.4)				
	175	0.305 (2.5)				
	180	0.679 (11)				
	184	0.708 (7.0)				
	185	0.755 (16)				
	186	0.236 (4.9)		0.236-1.192	0.703 ± 0.30 (43)	13
	187	1.102 (11)				
	188	0.503 (5.1)				
	191	1.192 (3.0)				
	192	0.542 (5.0)				
	193	1.096 (8.0)				
8Cl(12)	194	0.637 (1.3)	[d]			
	196	0.608 (3.9)				
	197	1.116 (9.0)				
	199	0.393 (6.3)		0.241-1.116	9.573 ± 0.26 (46)	8
	200	0.486 (6.8)				
	201	0.413 (5.3)				
	202	0.692 (5.9)				
	204	0.241 (9.1)				
9Cl(3)	206	0.431 (8.0)	[d]			
	207	0.066 (26)		0.066-0.565	0.354 ± 0.26 (73)	3
	208	0.565 (20)				

Table 3 (continued)

Homolog (No.)[a]	PCB Isomer No.[f]	RRF_i (%RSD)	Range	Mean ± S.D. (%RSD)	N[e]
10Cl(1)	209	0.418 (7.5) [d]	-	-	-

[a](No.) = number of theoretical isomers possible.

[b]Internal standard = 1,2-Dichloronaphthalene.

[c]Internal standard = 1,2,3,4-Tetrachloronaphthalene.

[d]Internal standard = Octachloronaphthalene.

[e]N = number of isomers studied; each isomer was measured in triplicate.

[f]See Ballschmitter and Zell 1980.

Table 4. Comparison of Relative Response Factors Between (GC)^2-ECD, GC-EIMS (Molecular Ion) and (GC)^2-NICIMS (m/z 35) for Homologous Series of PCBs

Homolog Series	(GC)^2-ECD[a] Range[d]	(GC)^2-ECD[a] Mean ± S.D. (%RSD)	N[c]	(GC)^2-NICIMS[a] Range[d]	(GC)^2-NICIMS[a] Mean ± S.D. (%RSD)	N	GC-EIMS[b] Range[e]	GC-EIMS[b] Mean ± S.D. (%RSD)	N
1Cl(3)	15.089-39.342	29.589 ± 12.78 (43)	3	0.456-1.787	0.924 ± 0.75 (81)	3	1.000-1.090	1.050 ± 0.84 (3.8)	3
2Cl(12)	0.425-10.641	4.271 ± 3.83 (90)	9	2.881-21.199	8.343 ± 6.17 (74)	8	1.000-2.062	1.736 ± 0.30 (17)	10
3Cl(24)	0.328-3.136	1.193 ± 0.68 (58)	9	0.721-10.901	2.921 ± 3.64 (125)	7	1.000-1.627	1.400 ± 0.24 (17)	9
4Cl(42)	0.385-2.229	1.074 ± 0.41 (38)	31	0.102-4.367	2.058 ± 1.02 (50)	16	1.000-2.146	1.549 ± 0.33 (21)	11
5Cl(46)	0.462-8.481	1.866 ± 1.39 (102)	35	0.465-1.216	0.805 ± 0.27 (33)	12	1.000-1.013	1.004 ± 0.01 (0.7)	3
6Cl(42)	0.391-1.912	0.873 ± 0.335 (35)	37	0.369-1.440	0.817 ± 0.29 (36)	16	1.000-1.321	1.153 ± 0.11 (9.6)	7
7Cl(24)	0.402-2.432	1.220 ± 0.419 (34)	21	0.236-1.192	0.703 ± 0.30 (43)	13	-	-	0
8Cl(12)	0.925-2.602	1.514 ± 0.679 (45)	10	0.241-1.116	0.573 ± 0.26 (46)	8	1.000-1.359	1.179 ± 0.25 (22)	2
9Cl(3)	1.005-1.816	1.391 ± 0.45 (35)	3	0.066-0.565	0.354 ± 0.26 (73)	3	-	-	0
10Cl(1)	-	1.168	1	-	0.418	1	-	-	0
	Overall:	0.328-39.342 (~120:1)		Overall:	0.066-21.199 (~320:1)				

[a] ECD data.

[b] Hertelli, et al., 1981.

[c] N = number of PCB isomers included in measurement.

[d] All values are relative to octachloronaphthalene.

[e] Responses were relative to lowest response for each group.

[f] ( ) = number of theoretical isomers possible.

homologous series when compared to ECD and NICI-MS. Some caution is needed in this comparison since the number of chlorobiphenyl isomers used in the study was small. This observation is consistent with the expectation that EI-MS produces the smallest variation of response between chlorobiphenyl isomers.

The extreme RRF values determined with ECD were approximately 120:1. This ratio would be somewhat larger if the calculations were made and expressed as RMRs (which includes M.W.). Nevertheless, the range of RRFs is not as large as reported by other investigators (Hutzinger et al. 1974, Boe and Egaas 1979, Albro et al. 1981, Safe et al. 1975). We believe that this is associated with differences between instrumental systems, since in our laboratory we have determined RRFs on three different (GC)-ECD systems (all with the same capillary column conditions), two of which are of the same make and model. We have observed significant differences in RRF values between all systems.

The extreme RMR (calculation includes M.W.) values determined with NICI-MS were approximately 320:1.

The importance of using appropriate response factors for quantifying individual chlorobiphenyl isomers cannot be overemphasized.

CHLOROBIPHENYL ISOMERS AND INSTRUMENT CALIBRATION

Primary Standards

The issue of instrument calibration for chlorobiphenyl isomer analysis in environmental, biological, and process stream samples has been recognised by investigators. As such, a program for the synthesis of the isomers was initiated.

Primary standards are needed for establishing reference data (spectral) banks for NICI-MS, EI-MS, FTIR, etc. for use in qualitative analysis or for verification purposes. They are needed also to establish relative retention indices for standardized (GC)[2] operating conditions.

The large scale synthesis of chlorobiphenyl isomers is obviously an expensive proposition, particularly if the supply must accommodate the needs of many analytical laboratories performing isomer quantification in environmental, biological and process stream samples. For this reason, our laboratories have been actively engaged in devising alternate techniques for instrument calibration.

Secondary Standards

Our objective has been to determine whether a secondary standard for calibrating instruments for quantification could be developed so that this secondary standard could be widely distributed among laboratories involved in chlorobiphenyl isomer analysis. The initial availability of the primary standards (209) is the key to developing a secondary standard.

Two approaches are currently under investigation in our laboratory. One utilizes the characterization of an Aroclor "cocktail" using the primary standards; the other employs a "clustering" algorithm to derive a small subset

of chlorobiphenyls from the 209 primary standards, with a chlorobiphenyl serving as a surrogate for several isomers. Each of these concepts is discussed here.

Characterization of Aroclor Mixtures

Since ample quantities of various commercial Aroclors are available for distribution, the possibility of preparing an Aroclor "cocktail" which is thoroughly characterized as to isomer speciation and quantity was undertaken.

An Aroclor 1016/1254/1260 mixture (2.5:2.0:1.0 w/w/w) was prepared. Table 5 lists the isomers tentatively identified in this mixture. Characterization has employed the use of relative retention time data for primary standards and matching with those in the Aroclor tripart mixture for three different $(GC)^2$-ECD systems and one $(GC)^2$-NICI-MS system. In addition, the molecular ions and mass spectra obtained from $(GC)^2$-NICI-MS and $(GC)^2$-EI-MS analysis of the Aroclor tripart mixture were used to establish the identity of the components in each of the chromatographic peaks. The peak number assignments in this table correspond to those given in Figures 9-11.

As indicated in Table 5 a majority of the chromatographic peaks have been assigned chlorobiphenyl isomer identities. In some cases the chromatographic peaks are heterogeneous and several isomers were identified. A few of the more toxic isomers (symmetrical 3,4 and 3,4,5) have been detected in this Aroclor mixture. A few peaks remain as unknowns; most are minor in quantity. As additional pure synthetic chlorobiphenyl isomers become available, the remaining unknowns will be identified, if possible. Aroclor 1016, 1221, 1242, 1254, and 1260 are also being characterized and will be reported elsewhere.

A complete Aroclor "cocktail" characterization (qualitatively and quantitatively) would allow its use as a secondary standard for instrument calibration provided that identical high resolution chromatographic conditions are employed in sample analysis as used for the Aroclor characterization.

Clustered Secondary Standard

Another concept being pursued is the development of a secondary standard which contains a small subset of individual chlorobiphenyl isomers that can be used to develop instrument responses for all PCBs of interest. The preliminary results of this approach are discussed here.

The response factors relative to the 1,2,3,4-tetrachloronaphthalene peak (TCN) for each PCB isomer were calculated using the expression given earlier.

The calculated response factors are listed in Table 6. From the 35 listed response factors, a calibration table was generated using a clustering algorithm based on the Student t-test.

Response factors were listed in increasing retention time order of the corresponding peaks. For each consecutive pair of response factors the Student t-test was performed:

Table 5. PCB Isomers with Retention Time Values Matching Peaks In Aroclor 1016/1254/1260 Mixture

Peak No.[a]	Chemical	Peak No.	Chemical
0	2	55	2,2',3,3',6
1		56	2,2',3,3,5',6
2	2,6 A/O 3 A/O 4	57	2,2',3,4,5',6
3	1,2-Dichloronaphthalene (i.s.)	58	2,2',3,4',5,6 A/O 3,3',4,4', A/O 2,2',3,3',5,5' A/O 2,2',3,4',5',6 A/O 2,2',3,4',5',6'
4	2,5		
5			
6		59	2,2',3,3',4,6 A/O 2,2',3,4,4',6 A/O 2,2',3,4,5,6
7	2,3		
8		60	2,2',3,3',4,6' A/O 2',3,4,5,5'
9		61	2,3,3',4,5
10	2,2',5 A/O 3,5	62	2,2',3,4,4' A/O 2,3,4,4',5
11		63	2,3',4,4',5
12		64	2,2',3,4,4',6,6'
13		65	2,3,4,4',5
14		66	
15	4,4'	67	
16	2,2',5,6'	68	2,3,3',4,4' A/O 2,3,3',4,5',6
17		69	2,2',3,4,5,5'
18	2,3',5	70	
19	2,2',3,6	71	2,2',3,4,4',5 A/O 2,3,3',4',5',6
20		72	
21	2,4',5	73	2,2',3,3',4,5
22	2,3,4	74	2,2',3,4,4',5 A/O 2,3,3',4,5,6 A/O 2,3,3',4',5,6
23	2',3,4		
24		75	
25	2,4,5 A/O 2,2',3,5	76	2,3,3',4,4',6
26	2,2',4,5' A/O 2,2',4,5	77	
27	2,2',3,5' A/O 2,3,5,6	78	2,2',3,3',4,6' A/O 2,2',3,4',5,5',6 A/O 2,2',3,4,4',5,6'
28	2,2',4,4' A/O 2,3,4,6		
29		79	2,2',3,4,5,5',6 A/O 2,2',3,4,4',5',6
30	2,4,4',6 A/O 2,2',3,4'	80	2,2',3,3',4,5,6'
31		81	2,2',3,3',5,5',6,6'
32	2,2',3,3'	82	2,2',3,3',4',5,6
33		83	2,2',3,4,4',5,6 A/O 2,2',3,3',4,5,6
34		84	2,3,3',4,5,5' A/O 2,2',3,3',4,5',6,6' A/O 2,2',3,3',4,4',6
35	1,2,3,4-Tetrachloronaphthalene (i.s.)		
36		85	2,2',3,4,4',5,6,6' A/O 2,3',4,4',5,5'
37	2,2',3,5,6 A/O 2,3',5,5'	86	2,2',3,3',4,5,6,6'
38	2,3',5',6	87	
39		88	
40		89	2,2',3,4,4',5,5' A/O 2,3,3',4',5,5',6
41	2,3',4',5 A/O 2,3,3',4'	90	2,3,3',4,4',5',6
42		91	2,2',3,3',4,4',5
43		92	2,2',3,3',4,5,5',6'
44		93	2,3,3',4,4',5,6
45	3,4,4',5 A/O 2,2',4,4',6,6' A/O 2,2',3,5,5'	94	2,2',3,3',4,4',5,6'
		95	
46	2,2',3,4',6,6'	96	3,3',4,4',5,5'
47	2,2',3,3',6,6'	97	2,2',3,3',4,4',5,6
48	2,2',4,5,5' A/O 2,2',3,4,4,6,6'	98	
49		99	
50	2,3,3',5,6 A/O 2,2',3,4,5	100	2,2',3,3',4,4',5,5'
51	2,3,3',6,6	101	
52	2,2',3,4,5'	102	2,2',3,3',4,4',5,5',6
53	2,3,4',5,6	103	Octachloronaphthalene (i.s.)
54			

[a]See Figures 9-11 for peak no. assignments.

A/O = and/or.

Table 6. Relative Response Factors for PCB Isomers

Peak No.[a]	PCB Isomer	RRF[b]	s[c]	n[d]
10	2,2',5 a/o 2,4,6	3.61	1.84	6
11	2,2',6,6'	7.69	0.71	3
18	2,2,5'	2.87	0.92	3
23	2',3,4	2.20	0.09	3
24	2,3,4'	0.76	0.03	3
25	2,2',5,5' a/o 2,4,5	3.61	0.67	6
27	2,2',3,5' a/o 2,2'3'5	2.98	0.26	3
30	2,4,4',6 a/o 2,2'3,4	2.30	0.51	6
37	2,2',3,5,6 a/o 2,3',5,5'	2.61	0.17	3
41	2,3',4',5	2.13	0.40	6
47	2,2',3,3',6,6'	5.30	1.57	3
48	2,2',4,5,5'	2.85	0.21	3
52	2,2',3,4,5'	1.81	0.13	3
53	2,3,4',5,6	2.22	0.16	3
58	2,2',3,4',5,6 a/o 3,3',4,4'	6.36	0.59	3
59	2,2',3,3',4,6	2.31	0.14	3
61	2,3,3',4,5	1.24	0.41	3
63	2,3',4,4',5	2.32	0.22	3
64	2,2',3,4,4',6,6'	2.13	0.50	3
68	2,3',4,4'	1.24	0.40	3
69	2,2',3,4,5,5' a/o 2,2',4,4',5,5',6	1.47	0.20	6
74	2,2',3',4,4',5	2.24	0.09	3
78	2,2',3,3',4,4', a/o 2,2',3,4',5,5',6	1.66	0.15	3
80	2,2',3,3',4,5,6' a/o 2,2',3,4,5,5',6	1.11	0.14	3
81	2,2',3,3',5,5',6,6'	2.07	0.56	3
82	2,2',3,3',4',5,6	2.08	1.13	3
84	2,2',3,3',4,5',6,6'	3.51	0.35	3
85	2,2',3,4,4',5,6,6'	1.29	0.07	3
86	2,2',3,3',4,4',6,6' a/o 2,2',3,3',4,5,6,6'	1.34	0.34	3
89	2,2',3,4,4',5,5' a/o 2,3,3',4',5,5',6	1.58	0.55	6
90	2,3,3',4,4',5',6	1.07	0.33	3
93	2,2',3,3',4,5',6	0.34	0.13	3
96	2,2',3,3',4,5,5',6,6'	1.27	0.39	9
98	2,2',3,3',4,4',5,6,6'	4.47	1.00	3
100	2,2',3,3',4,4',5,5'	1.27	0.18	3

[a] Peak number as in Figure 9-11.
[b] RRF = relative response factor.
[c] s = standard deviation.
[d] n = sample size.

$$t = (RRF_i - RRF_{i+1}) \cdot \bar{s}^{-1} \cdot (1/n_i + 1/n_{i+1})^{-1/2}$$

where t is the Student t value; RRF_i and RRF_{i+1} were response factors for sequential peaks; $\bar{s}$ is pooled standard deviation, and n_i and n_{i+1} are number of runs. Pooled standard deviation was calculated as:

$$\bar{s} = \frac{(n_i - 1) \cdot s_i^2 + (n_{i+1} - 1) \cdot s_{i+1}^2}{n_i + n_{i+1}^{-2}}$$

where s_i and s_{i+1} were standard deviation of RRF_i and RRF_{i+1}, respectively.

If the calculated t was lower than t_{cr} (taken from Student t tables) for $n_i + n_{i+1}$ degrees of freedom and $\alpha = 0.95$, the two response factors were replaced by a common mean (RRF_j).

$$RRF_j = \frac{n_i \cdot RRF_i + n_{i+1} \cdot RRF_{i+1}}{n_i + n_{i+1}}$$

$$s_j = \bar{s} \times \frac{n_i + n_i + 1}{n_i + n_{i+1}^{-2}}$$

where s_j is the standard deviation for RRF_j.

After all pairs were tested, the clustering procedure was repeated to generate a second calibration table. This procedure was continued until response factors were statistically different from the two nearest values. The clustered list of response factors is presented in Table 7.

Chromatographic peaks then are identified as PCB isomers by matching retention time with an Aroclor calibration mixture.

Sample components with retention times matching peaks in the Aroclor standard mixture were identified as PCBs in the samples. Each matched peak was calculated as:

$$PCB_i = RRF_i \times Amt_{IS} \times \frac{A_i}{A_{IS}}$$

If the PCB peak does not correspond to a calibrated peak, the response factor of the nearest calibrated peak was used. The "total PCB value" for the sample then was given by:

$$PCB = \Sigma\ PCB_i$$

Table 7. Clustered List of Relative Response Factors

Peak No.	RRF	s	n
10	3.61	1.84	6
11	7.69	0.71	3
18-23	2.54	0.45	6
24	0.76	0.03	3
25-27	3.40	0.55	9
30-41	2.29	0.39	15
47-48	4.08	1.00	6
52	1.81	0.19	3
53	2.22	0.16	3
58	6.36	0.59	3
59	2.31	0.14	3
61	1.24	0.41	3
63-64	2.23	0.35	6
68-69	1.39	0.25	9
74-82	1.79	0.42	21
84	3.51	0.35	3
85-90	1.37	0.38	15
93	0.34	0.13	3
96	1.27	0.39	9
98	4.47	1.00	3
100	1.27	0.18	3

An example of the use of this concept has been applied to quantifying the total PCB weight for an Aroclor 1016/1254/1260 mixture using $(GC)^2$-ECD:

Observed weight: 562
Actual weight (gravimetrically determined): 550

$$\text{Error} = \frac{562-550}{550} \times 100 = 2.2\%$$

By expanding the "clustering" algorithm analysis of responses to include all the available chlorobiphenyl isomers, a surrogate composition containing a small number of isomers (20-40?) will be defined. Thus, only this set of chlorobiphenyl isomers needs to be synthesized on a large scale.

APPLICATION OF STATE-OF-THE-ART METHODS TO PCB ANALYSIS IN ENVIRONMENTAL AND BIOLOGICAL MATRICES

With these recent parallel developments laboratories have begun to characterize and quantify chlorobiphenyl isomers in environmental, biological, and process stream samples. Examples of the combined state-of-the-art technology described above are discussed here.

The benefit of $(GC)^2$ over conventional packed column gas chromatography for analysis of sera and adipose is exemplified by Figures 14 and 15, respectively. Figure 14 depicts the profile for a human serum sample extract chromatographed on a packed column (top) and capillary (bottom). Improved resolution and hence better signal to noise is realized with $(GC)^2$. Figure 15 shows that the low resolution profile has many hidden isomers in its chromatographic peaks which are revealed by $(GC)^2$.

Identification of specific chlorobiphenyl isomers in human tissues may be important for two reasons: for assessment of long-term persistence and for evaluation of potential health effects as suggested by toxicological studies on individual isomers. Recently, the disposition of PCB isomers in occupationally exposed persons has been reported (Wolff et al. 1982). The concentrations of PCBs in adipose and plasma were related to duration and intensity of exposure in the workplace. It was reported that PCB levels in adipose tissue were proportional to those in plasma (total PCB ratio of 190:1). The distribution of specific chlorobiphenyl isomers between plasma and adipose tissue was reported, however, to be related to specific ring position substitution, differing among isomers.

Similar observations have been reported in our laboratory. For these reasons, and because of a limited number of isomers and suboptimal analytical conditions in the recent study, research was undertaken to characterize the individual isomers in sera and adipose of occupationally exposed individuals using state-of-the-art techniques. Using approximately 165 primary reference standards (individual PCB isomers) and $(GC)^2$-NICI-MS (RRT and molecular ions) the isomers were tentatively identified (Tables 8 and 9). Also the detected and identified peaks were referenced against an Aroclor 1016/1254/1260 standard mixture.

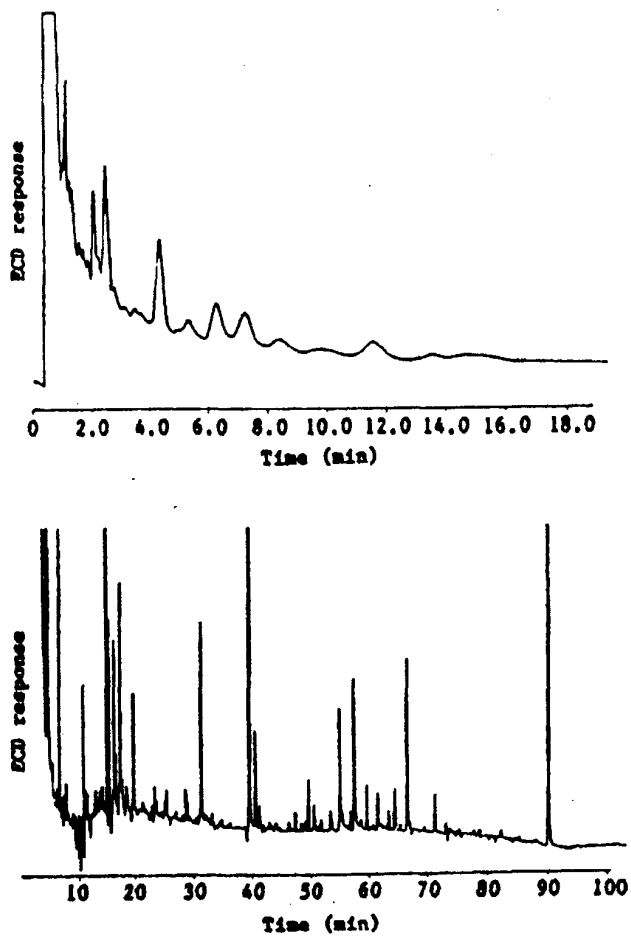

Figure 14. Chromatograms of human serum sample.
Top - packed column, bottom - capillary.

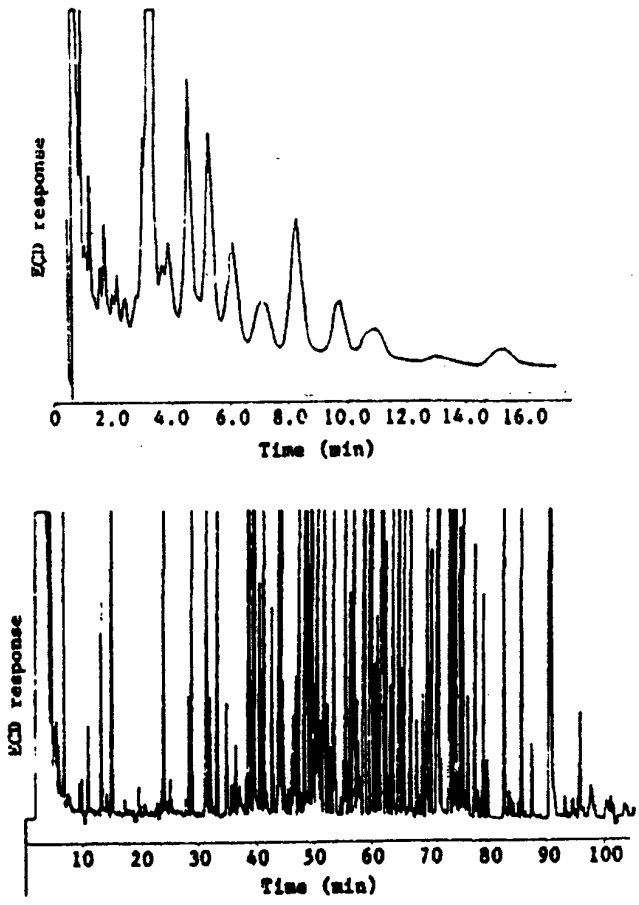

Figure 15. Chromatograms of human adipose tissue. Top - packed column, bottom - capillary.

Table 8. Individual PCB Isomers Tentatively Identified in Human Serum

1,2-Dichloronaphthalene	1,2,3,4-Tetrachloronaphthalene	Octachloronaphthalene		Corresponding Aroclor
Mean RRT (%RSD)	Mean RRT (%RSD)	Mean RRT (%RSD)	Chlorinated Chemical	Peak No.[a]
1.0[illegible] (0)	0.423 (0.27)	0.200 (0.29)	1,2-Dichloronaphthalene	3
2.[illegible]0 (0.02)	1.000 (0)	0.472 (0.12)	1,2,3,4-Tetrachloronaphthalene	35
5.0[illegible] (0.20)	2.120 (0.03)	1.000 (0)	Octachloronaphthalene	103
2.649 (0.31)	1.036 (0.20)	0.489 (0.12)	2,3',5,5' A/O 2,2',3,5,6	37
2.767 (0.19)	1.171 (0)	0.552 (0.11)	2,2',4,5,5', A/O 2,2',3,4,6,6'	48
2.847 (0.18)	1.305 (0)	0.560 (0)	2,3,3',4,6	51
2.861 (0.21)	1.211 (0.05)	0.571 (0)	2,2',3,4,5'	52
2.882 (0.20)	1.219 (0.13)	0.575 (0)	DDE	
2.993 (0.21)	1.261 (0)	0.585 (0)	?	54
3.003 (0.15)	1.270 (0.09)	0.599 (0.10)	2,2',3,5,5',6	56
3.042 (0.20)	1.304 (0.04)	0.613 (0)	2,2',3,4',5,6 A/O 2,3',4,4' A/O 2,2',3,4,5,6' A/O 2,2',3,3',5,5' A/O 2,3',3,4',5',6	58
3.102 (0.20)	1.346 (0.04)	0.635 (0)	2,2',3,3',4,6' A/O 2',3,4,5,5'	60
3.259 (0.24)	1.379 (0.08)	0.651 (0.09)	2,3',4,4',5 A/O 2',3,3',4,5	63
3.353 (0.18)	1.418 (0.04)	0.669 (0)	2,3,3',4,4', A/O 2,3,3',4,5',6	68
3.405 (0.25)	1.441 (0)	0.680 (0.09)	2,2',3,4,5,5'	69
3.443 (0.25)	1.547 (0.07)	0.687 (0.08)	?	70
3.463 (0.17)	1.466 (0.04)	0.691 (0.08)	2,2',3,4,4',5 A/O 2,3,3',4',5',6	71
3.452 (0.20)	1.474 (0.04)	0.695 (0)	2,2',3,3',4,5	73
3.481 (0.22)	1.481 (0.04)	0.699 (0)	2,2',3,4,4',5' A/O 2,3,3',4,5,6 A/O 2,3,3',4',5,6	74
3.541 (0.24)	1.499 (0.08)	0.707 (0)	2,3,3',4,4',6	76
3.600 (0.20)	1.523 (0.04)	0.718 (0)	2,2',3,3',4,4', A/O 2,2',3,4',5,5',6 A/O 2,2',3,4,4',5,6'	78
3.657 (0.22)	1.547 (0.04)	0.738 (0)	2,2',3,4,5,5',6 A/O 2,2',3,4,4',5',6 A/O 2,2',3,3',4,5,6'	79,80
3.705 (0.23)	1.568 (0.04)	0.740 (0.00)	2,2',3,3',4',5,6	82
3.761 (0.17)	1.591 (0.04)	0.751 (0.08)	2,2',3,4,4',5,6 A/O 2,2',3,3',4,5,6	83
3.825 (0.22)	1.619 (0.08)	0.764 (0.08)	2,2',3,4,4',5,6,6' A/O 2,3',4,4',5,5'	85
3.916 (0.20)	1.657 (0.04)	0.782 (0)	?	87
4.013 (0.19)	1.699 (0.07)	0.801 (0)	2,2',3,4,4',5,5' A/O 2,3,3',4',5,5',6	89
4.109 (0.20)	1.739 (0.03)	0.820 (0)	2,2',3,3',4,4',5	91
4.891 (0.21)	1.943 (0.03)	0.916 (0)	2,2',3,3',4,4',5,5'	100

[a] Aroclor 1016/1254/1260 mixture, see Figures 9-11.

Table 9. Individual PCB Isomers Tentatively Identified in Human Adipose Tissue

1,2-Dichloronaphthalene	1,2,3,4-Tetrachloronaphthalene	Octachloronaphthalene		Corresponding Aroclor[a] Peak No.
Mean RRT (1968)	Mean RRT (1968)	Mean RRT (1968)	Chlorinated Chemical	
1.000 (0)	0.424 (0.14)	0.201 (0.27)	1,2-Dichloronaphthalene	3
1.357 (0.06)	1.000 (0)	0.473 (0.12)	1,2,3,4-Tetrachloronaphthalene	15
4.966 (0.12)	3.518 (0.05)	1.000 (0)	Octachloronaphthalene	101
0.893 (0.09)	0.379 (0.15)	0.179 (0)	?	-
1.322 (0.04)	0.943 (0.09)	0.305 (0.19)	?	-
1.756 (0.06)	0.745 (0.08)	0.353 (0)	?	-
1.783 (0.06)	0.757 (0.08)	0.358 (0.16)	?	-
1.949 (0.11)	0.827 (0.07)	0.391 (0.15)	2',3,6	13
1.191 (0.10)	0.893 (0.11)	0.422 (0.14)	2,6,6 A/O 2,2',3,5	15
2.442 (0.19)	1.036 (0.06)	0.490 (0)	2,3',3,5' A/O 2,2',3,5,6	17
2.538 (0.06)	1.072 (0.08)	0.507 (0)	?	20
2.578 (0.10)	1.093 (0.05)	0.517 (0)	2,3,3',6' A/O 2,3',4',5	41
2.603 (0.10)	1.104 (0.05)	0.522 (0)	?	42
2.623 (0.17)	1.113 (0.10)	0.526 (0.11)	?	43
2.702 (0.09)	1.150 (0)	0.544 (0.11)	2,2',3,3',6,6'	47
2.750 (0.09)	1.170 (0)	0.553 (0)	2,2',3,3,5' A/O 2,2',3,4,6,6'	48
2.814 (0.11)	1.194 (0)	0.564 (0)	2,3,3',5,6 A/O 2,2',3,4,5	50
2.839 (0.11)	1.204 (0)	0.569 (0)	2,3,3',4,6	51
2.851 (0.12)	1.209 (0.05)	0.572 (0)	2,2',3,4,5'	52
2.873 (0.12)	1.219 (0)	0.576 (0)	[redacted]	-
2.922 (0.10)	1.240 (0.05)	0.586 (0)	?	54
2.990 (0.16)	1.268 (0.08)	0.600 (0.10)	2,2',3,5,5',6	54
3.071 (0.11)	1.303 (0.04)	0.616 (0)	2,2',3,4',5,6 A/O 2,3',4,5' A/O 2,2',3,4,5,6' A/O 2,2',3,3',5,5' A/O 2,2',3,4',5',6	58
3.171 (0.13)	1.346 (0.04)	0.636 (0)	2,2',3,3',4,6' A/O 2',3,4,5,5'	60
3.210 (0.13)	1.366 (0.04)	0.645 (0)	2,3,3',4,5	61
3.248 (0.13)	1.378 (0.04)	0.651 (0)	2,3',4,4',5	63
3.330 (0.14)	1.416 (0.04)	0.669 (0)	2,3,3',4,4' A/O 2,3,3',4,5',6	66
3.398 (0.11)	1.439 (0)	0.680 (0)	2,3',3,4,5,5'	69
3.438 (0.15)	1.454 (0.08)	0.687 (0.08)	?	70
3.451 (0.11)	1.464 (0.04)	0.692 (0)	2,3',3,4,4',5 A/O 2,3,3',4',5',6	73
3.486 (0.13)	1.479 (0.04)	0.699 (0)	2,3',3,4,4',5' A/O 2,3,3',4,5,6 A/O 2,3,3',4',5,6	74
3.498 (0.13)	1.483 (0.03)	0.701 (0)	?	-
3.508 (0.13)	1.487 (0.07)	0.703 (0)	?	75
3.526 (0.10)	1.496 (0)	0.707 (0)	2,3,3',4,4',6	76
3.572 (0.14)	1.515 (0.06)	0.716 (0.06)	?	-
3.596 (0.13)	1.525 (0.07)	0.720 (0)	2,3',3,4',5,5',6 A/O 2,3',3,3',4,4' A/O 2,3',3,4,4',5,6'	78
3.643 (0.12)	1.545 (0.04)	0.730 (0.08)	2,3',3,4,5,5' A/O 2,3',3,4,4',5',6 A/O 2,3',3',4,5,6'	79.80
3.673 (0.10)	1.558 (0.06)	0.736 (0.08)	2,2',3,3',3,5',6,6'	81
3.690 (0.12)	1.566 (0.06)	0.740 (0)	2,3',3,3',4',5,6	82
3.747 (0.12)	1.590 (0.06)	0.751 (0)	2,3',3,4,4',5,6 A/O 2,3',3,3',4,5,6	83
3.810 (0.10)	1.616 (0.09)	0.764 (0)	2,3',3,4,4',5,5,6' A/O 2,3',4,4',5,5'	86
3.890 (0.09)	1.650 (0)	0.780 (0)	?	-
3.890 (0.16)	1.654 (0.06)	0.782 (0)	? (OC1)	87
3.937 (0.13)	1.670 (0.06)	0.790 (0)	? (PC1)	88
3.996 (0.14)	1.695 (0.06)	0.801 (0)	2,3',3,4,4',5,5' A/O 2,3,3',4',5,5',6	89
4.092 (0.12)	1.735 (0.03)	0.820 (0)	2,3',3,3',4,4',5	91
4.129 (0.15)	1.752 (0.07)	0.828 (0)	2,2',3,3',4,5,5',6'	92
4.152 (0.13)	1.761 (0.03)	0.833 (0)	2,3,3',4,4',5,6	93
4.186 (0.10)	1.776 (0)	0.840 (0.07)	2,2',3,3',4,4',5,6'	94
4.213 (0.14)	1.787 (0.06)	0.845 (0)	? (OC1)	95
4.262 (0.17)	1.808 (0)	0.854 (0.08)	?	96
4.314 (0.13)	1.830 (0.08)	0.866 (0.07)	? (OC1)	97
4.340 (0.13)	1.930 (0.05)	0.916 (0)	2,2',3,3',4,4',5,5'	100
			2,3',3,3',4,4',5,5',6	103

[a]Aroclor 1016/1254/1260 mixture, see Figures 9-11.

Table 10 provides a composite listing of PCB isomers tentatively identified in 4 sera and 4 adipose of this population under study. The presence of the symmetrical 3,4,5 and 3,4 isomers was tentatively established. These two isomers are presumably amongst the most toxic of the PCB isomers. The quantification of the individual isomers in this set of about 100 individuals will be reported elsewhere.

These state-of-the-art techniques are currently being applied to the analysis of samples of rain, surface water, sediment, fish, human mother's milk, maternal cord blood, etc. (Mullin 1981). Figures 16-21 depict examples of profiles obtained for specific cases cited.

Finally, an area of considerable activity involves the development of computerized data analysis systems. With the use of automated GCs, a considerable amount of data is generated when using $(GC)^2$. Computerization is being done not only to facilitate calculations of PCB isomer and total PCB levels in samples, but is also being developed to assist in answering comparative questions about samples (Stalling et al. 1982). Questions relating to biotransformation, distribution and fate through an ecosystem and sources require sophisticated pattern recognition techniques for comparing and relating PCB information between environmental and biological samples (Stalling et al. 1982).

SUMMARY

Significant studies have been performed during the past 3-4 years in developing: (a) high resolution GC capillaries tailor-made for PCB analysis; (b) ultra-sensitive electron capture detectors compatible with capillary column flow rates; (c) an expanded linear dynamic range of operation for the ECD; (d) the operating conditions for NICI-MS necessary to measure and characterize PCBs in environmental and biological samples; and (e) the development of primary and secondary PCB standards for calibration of instrumentation. The research in the latter area continues as more work needs to be done. Investigators are just beginning to reap the fruits of their labor by applying this advanced analytical methodology to real world problems.

Table 10. Tentatively Identified PCB Isomers Detected in Four Human Sera and Four Adipose Samples by $(GC)^2$/ECD and $(GC)^2$/NICI-MS/DS

PCB Isomer	Corresponding Aroclor Peak No.[a]
	12
2,2',6,6' A/O 3,3'	-
2',3,4	23
2,4,5 A/O 2,2',3,5	25
2,4,6',6 A/O 2,2',3,4'	30
2,2',3,5,6 A/O 2,3',5,5'	37
	39
	40
2,3',4',5 A/O 2,3,3',4'	41
-	42
-	43
2,2',3,3',6,6'	47
2,2',4,5,5' A/O 2,2',3,4,6,6'	48
2,3,3',5,6 A/O 2,2',3,4,5	50
2,3,3',4,6	51
2,3',3,4,5'	52
-	54
2,2',3,5,5',6	56
2,2',4,4',5,6'	-
2,2',3,4',5,6 A/O 3,3',4,4', A/O 2,2',3,3',5,5' A/O 2,2',3,4',5',6 A/O 2,2',3,4,5,6'	58
2,2',3,3',4,6 A/O 2,2',3,4,4',6 A/O 2,2',3,4,5,6	59
2,2',3,3',4,6' A/O 2',3,4,5,5'	60
2,3,3',4,5	61
2,3',4,4',5	63
2,3,3',4,4' A/O 2,3,3',4,5',6	68
2,2',3,4,5,5'	69
-	70
2,2',3,4,4',5 A/O 2,3,5',4',5',6	71
2,2',3,3',4,5	73
2,2',3,4,4',5' A/O 2,3,3',4,5,6 A/O 2,3,3',4',5,6	74
-	75
2,3,3',4,4',6	76
2,2',3,3',4,4' A/O 2,2',3,4',5,5',6 A/O 2,2',3,4,6',5,6'	78
2,2',3,4,5,5',6 A/O 2,2',3,4,4',5',6 A/O 2,2',3,3',4,5,6'	79,80
2,2',3,3',5,5',6,6'	81
2,2',3,3',4',5,6	82
2,2',3,4,4',5,6 A/O 2,2',3,3',4,5,6	83
2,2',3,4,4',5,6,6' A/O 2,3',4,4',5,5'	85
-	87
-	88
2,2',3,4,4',5,5' A/O 2,3,3',4',5,5',6	89
2,2',3,3',4,4',5	91
2,2',3,3',4,5,5',6'	92
2,3,3',4,4',5,6	93
2,2',3,3',4,4',5,6'	94
-	95
3,3',4,4',5,5'	96
2,2',3,3',4,4',5,6	97
2,2',3,3',4,4',5,5'	100
2,2',3,3',4,4',5,5',6	102

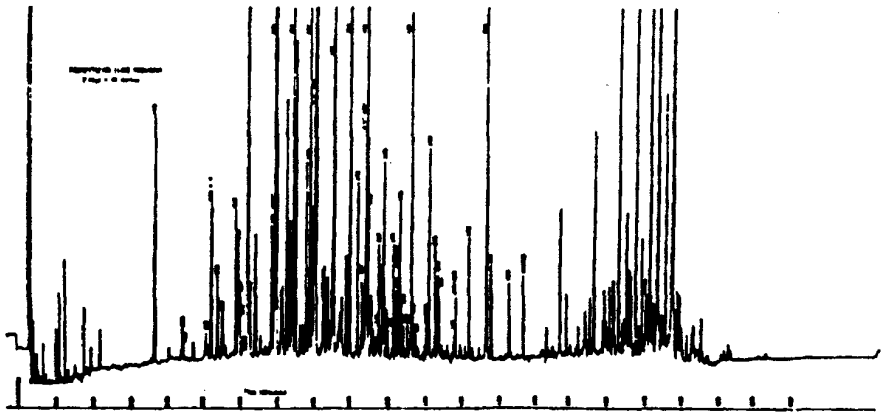

Figure 16. Chromatogram of precipitation sample showing PCBs and PCTs.

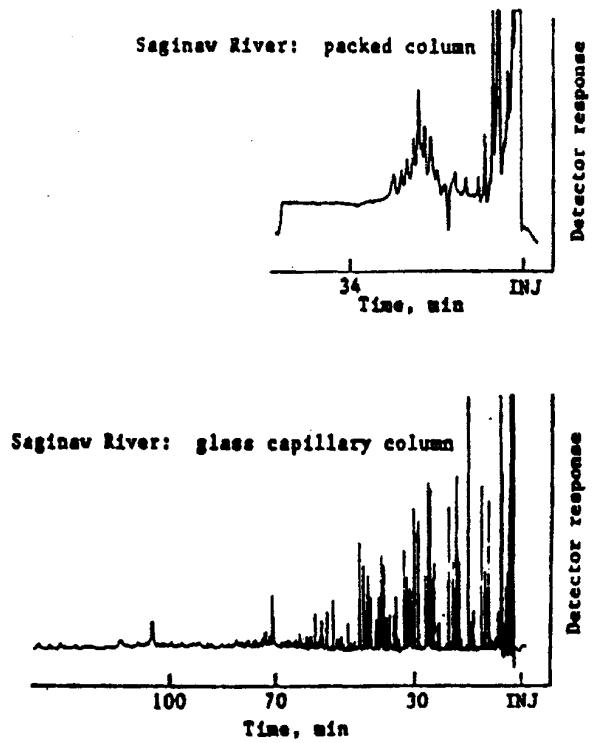

Figure 17. Chromatograms of surface water sample.
Top - packed column, bottom - capillary.

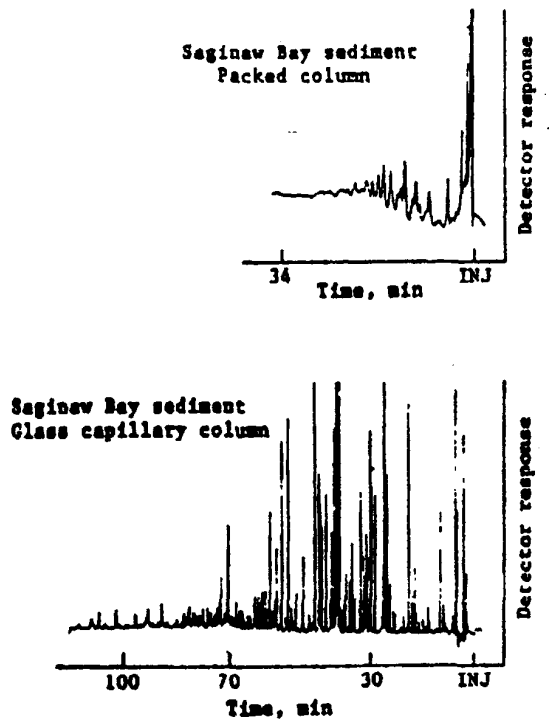

Figure 18. Chromatograms of sediment sample.
Top - packed column, bottom - capillary.

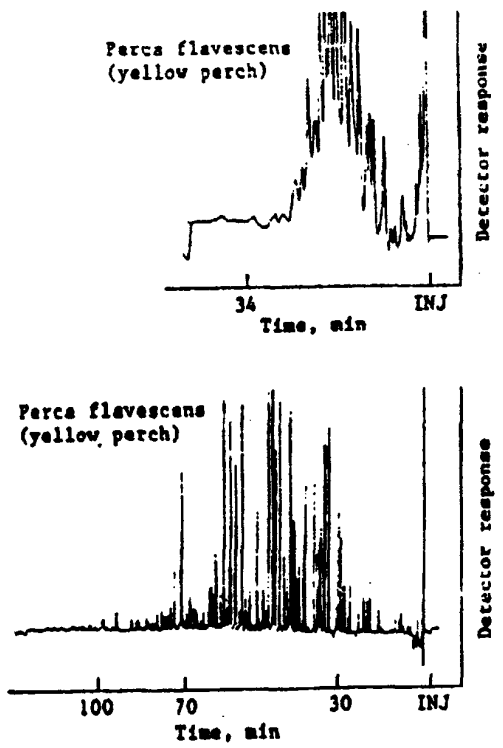

Figure 19. Chromatograms of fish sample.
Top - packed column, bottom - capillary.

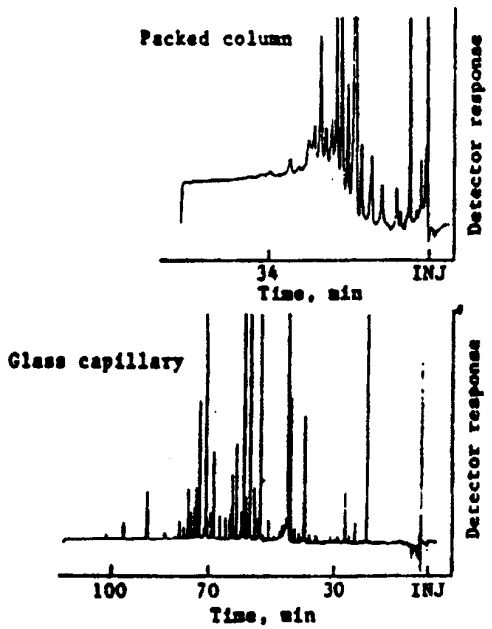

Figure 20. ..Chromatogram of human mother's milk.
Top - packed column, bottom - capillary.

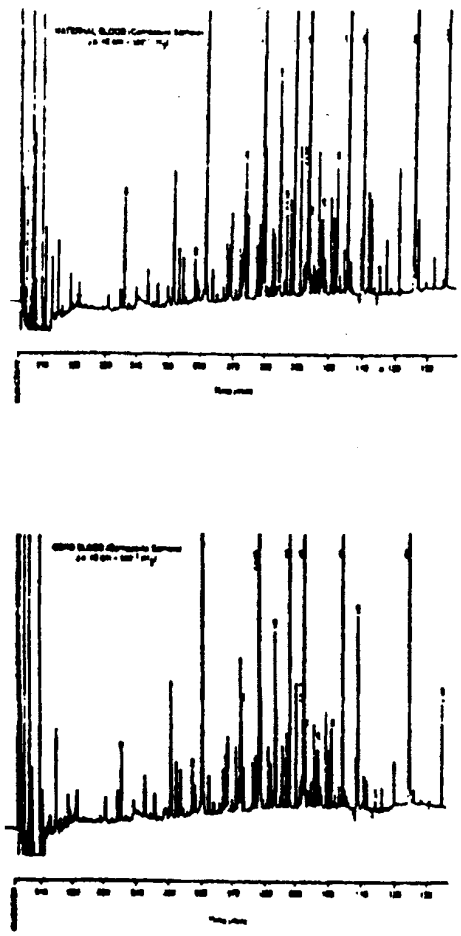

Figure 21. Chromatograms of blood sample. Top - maternal, Bottom - cord (Matched pair).

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DISCUSSION SUMMARY

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The paper by Dr. Pellizzari described the recent advances in instrumental measurement techniques for PCBs, which allow identification of individual PCB isomers at low levels of detection. The discussion period centered on the feasibility and desirability of using such techniques in routine laboratory analysis of environmental samples. The relevance of isomer specificity to current concepts of biological activity of PCBs was thought to be of potential significance. The utilization of high resolution gas chromatography (HRGC) was stressed as a powerful tool in elucidation of structure activity relationships. In addition, the matter of method validation was discussed, with reference to the importance of comparing data from various laboratories for PCBs in environmental samples. The currently developing area of chemometrics, and its application to pattern recognition of PCBs in environmental samples was identified as an important new contribution to the field.

The advantages of HRGC in PCB analysis include the specificity and sensitivity of the method. Specificity is achieved by resolution, which allows differentiation of PCBs from interferents such as pesticide residues. These analyses may be conducted routinely using electron capture detection (EC), but additional specificity may be introduced by using various mass spectrometric detection techniques.

In particular, HRGC was perceived to be a significant improvement over packed column GC (LRGC) for determination of lower chlorinated biphenyls (LPCB). It was emphasized that the electron capture response for LPCBs, depending both on degree of substitution and on orientation of substitution, could vary several orders of magnitude. In general, the LPCBs have a much lower response than the higher chlorinated PCB congeners (HPCB), so that lack of sensitivity may cause them to be overlooked. One investigator was skeptical of LPCB analysis using HRGC - EC. Further, the pattern of LPCB in environmental samples may not be recognizable in comparison with the usual Aroclor standards. Whereas HPCB, using either LRGC or HRGC, can generally be matched approximately to an Aroclor standard, the LPCBs are often so extensively degraded that visual pattern recognition is impossible.

The utility of HRGC in routine analysis was suggested to be feasible in the immediate future by use of short columns and autosampling devices. The use of HRGC as an ancillary tool for qualitative identification of PCB congeners, with subsequent extrapolation to larger numbers of samples analyzed by LRGC, was urged by several participants. Although the reliability of this approach was objected to by some of the discussants, its practicability was thought to have some basis in the past literature. In several past studies, identification of individual PCB congeners in human or animal tissues was reported. Some investigators used HRGC as an adjunct to LRGC analyses, while in other cases use of efficient packed columns, often in combination with

specific sample preparation techniques, allowed identification of individual components. For example, non-ortho, mono-, and di- orthochloro substituted PCBs have been reported to be separable by column chromatography on charcoal.

The sensitivity of HRGC was reported to be in the range of 0.1-100 pg depending on the particular PCB isomer and on the method of detection. Using mass spectrometry as the detection method (MS), greater sensitivity can be achieved using single ion monitoring than using full scan. The importance of coordinating preparative methodology with instrumental methodology was stressed. It was pointed out that the achievement of low limits of detections requires validation of clean-up methodology as well as instrumental detection. That is, instrumental detection of 1 pg must be combined with a sample clean-up method that permits recovery of that amount of material in order to specify a limit of detection of 1 pg for analysis of actual biological samples.

The application of HRGC to identification and quantitation of PCB derived degradation products, especially in the presence of PCBs, was discussed briefly. In particular, the use of LRGC to identify PCDFs in PCB mixtures was mentioned. Several such reports exist in the recent literature. The validity of electron capture with either LRGC or HRGC to identify PCDFs or PCDDs in the presence of PCBs was questioned. Dr. Stalling pointed out that in analysis of soot from a transformer fire, the presence of large amounts of chlorinated biphenylenes had prevented meaningful determination of PCDFs by EC detection. Recent papers have reported the synthesis of a number of PCDF isomers as standards, as well as identification and quantitation of these compounds in environmental samples using HRGC in combination with MS detection, following a highly specific clean-up procedure. Sensitivities were in the same range as for PCB isomers.

Dr. Stalling also mentioned on-going research into a new detection system for PCBs, which employs a halogen specific helium plasma detector, which may permit differentiation of brominated and chlorinated biphenyls, for example.

The major problems and disadvantages for widespread application of HRGC to PCB analysis were discussed. The most common drawbacks mentioned were the cost and time of analysis, especially in the use of MS as the method of detection, for routine analysis of PCBs. Investigators involved in epidemiologic investigations were doubtful of the practicality of HRGC analysis of large numbers of samples.

The lack of standards, available to all investigators, is a current problem. Although several laboratories have synthesized a large number of individual PCB congeners, the availability through commercial sources is limited. The uniformity of purity of standards is also important. Even with Aroclors or other commercial PCB mixtures, the PCB content of individual congeners may vary from batch to batch. That several major discrepancies existed in recent reports of individual PCB congeners in Aroclors or in biological samples was pointed out. PCB congeners reported from at least four different laboratories were different even for commercial PCB mixtures (e.g. Aroclors) within the past few years, and it was strongly suggested that this point be clarified. The possibility of quantitation using proportions of individual PCB congeners in Aroclors (along the lines of the method of Webb

and McCall as used for LRGC) has become a possibility with the publication of such data in the past few years.

The necessity of conducting interlaboratory studies was stressed, as part of the quality control and method validation of sample analysis, whatever the method of PCB identification and quantitation. In particular, as previously mentioned, the importance of introducing such programs into analytic methodologies now developing for HRGC analysis, was strongly emphasized. Quality control should apply to clean-up procedures, to quantitation procedures, and to method validation.

The lack of homogeneity of PCB analytic procedures reported in the literature was suggested by results of interlaboratory studies at CDC, where quantitation was achieved using LRGC. Dr. Larry Needham reported that one interlaboratory study involved analysis by various laboratories, using any method of their choice, of blood serum from cattle fed PCBs. The results, as described by Dr. Needham, resembled a random assortment of numbers. When the laboratories were later asked to use a selected method, the results were much improved, but still had considerable variance.

Dr. Susan Sonchick (Versar, Inc.) reported that twenty laboratories had recently participated in analysis of PCBs in oil matrices using LRGC. According to Dr. Sonchick, several standardized clean-up procedures were specified for different oils. The results should be available for evaluation within a few months.

Dr. Carl Manger (Baltimore Gas and Electric Co.) reported that the ASTM would soon publish a method for analysis of Aroclor mixtures in oil (1983 ASTM part 40), which was also evaluated in an interlaboratory study.

In terms of recent advances, little progress was thought to have been made in validation and standardization of clean-up methods for biological materials. Two papers by Albro in the past three years have emphasised the wide variability incorporated into sample preparation procedure from methods of extraction and clean-up (acid wash, column chromatography, HPLC). Implicit in such variability is the important factor of differential extraction and recovery of different PCB isomers. The critical importance of extraction efficiency of different techniques was also pointed out in a recent publication, where levels of 20-3000 ppb of PCDDs and PCDFs were observed in identical fly-ash samples using seven different extraction methods. The validity of quality control methods which employ fortified tissue samples for recovery determinations has also been questioned by Albro, who has compared samples fortified with chemical in different solvents to samples having endogenous C14 - labelled chemical. The importance of reliability versus recovery efficiency has also been mentioned by Albro. The use of automated gel permeation chromatography for removal of lipid has been used by several laboratories, and is advantageous because it can be automated, recoveries are quantitative, and lipid is well separated from PCB residues. However, it was pointed out that the reliability of this method, in one report, was poorer than another widely used technique (acetonitrile-hexane partition), although the latter had slightly lower average recoveries.

It was emphasized that in addition to the variance introduced by sample preparation techniques, the reliability of PCB quantitation has been reported to be no better than a factor of three to ten, so that the combination of such variances must inevitably lead to wide interlaboratory variance, as underscored by Dr. Needham's remarks.

The question of reliability was raised with reference to enforcement technology, where levels of 50 ppm in certain oils are subject to control. The analytic variance, as well as the sensitivity, of a given preparative and instrumental technique are factors to be considered in setting realistic control guidelines.

The toxicological significance of identifying individual PCB congeners in environmental samples was discussed. In view of the findings presented in other sessions of the symposium, many of which have also been reported in the literature, the consensus recognized the importance of PCB isomer types. The measurement of PCBs in human blood for example, has been performed on the premise that intensity of exposure, as reflected in concentration of total PCBs, would be related to toxic effects. Thus, with very few exceptions, PCB residues in human tissues have been reported as a total concentration, or the sum, of several LRGC peaks. Although in a few studies, individual PCB congeners have been identified, population base studies invariably have reported a total number, on which biological correlates have been based. In several cases, not even the type of Aroclor used to estimate PCBs has been specified in such studies. It was pointed out that such discrepancies, along with the methodological insufficiencies also mentioned, cast doubt on the comparability of such studies, in terms of PCB exposure or in terms of biological correlates. Clearly, even the LRGC techniques currently in use require standardization to provide some consistency among different studies, both for quantitation and for designation of the kind of PCB exposure. Although the state-of-the-art of PCB analysis would dictate use of HRGC, with subsequent biological correlation with individual isomers, the advocates of this methodology were consistently reminded of the rapidity and large volume of samples which must be dealt with in many studies. The application of pattern recognition to HRGC analysis was recommended, and it was further discussed whether this computer method would be applicable to LRGC. The use of HRGC to complement large numbers of LRGC was also recommended. Although presently limited for the quantitative evaluation of large numbers of samples, HRGC was urged as an area of further development for routine usage in combination with pattern recognition techniques. It was suggested to be a potential asset in identifying PCB congener subsets for use in predicting relative toxicity.

One major shortcoming of LRGC, in estimation of PCBs, was said to be its failure to detect or to recognize lower chlorinated congeners. Usually, an analyst attempts to match a sample with an Aroclor mixture, which is then used for quantitation. Both the lack of detection (low detector response) and the purported underestimate of concentration (use of standards unsuited to less chlorinated isomers) were cited as contributory factors. However, two recent papers, one with humans and one with animals, reported comparable LRGC and HRGC values. The use of a method such as the commonly cited Webb and McCall automatically incorporates a compensatory factor for lower response of LPCBs.

Nevertheless, the advantages of HRGC analysis for LPCB were impressive, showing greater sensitivity and discrimination of interferents.

The developing chemometric theory and its application to PCB pattern recognition was summarized briefly by Dr. Stalling. In collaboration with specialists in this field, his group has applied a statistical method (principal component analysis) to 69 PCB congeners in HRGC analyses of approximately 1,000 environmental samples. The results are presented in an eigen vector format, or principal component projection, which allows the analyst to determine the behavior of individual PCB components within a similar type or class of samples. Some of his findings will be presented in a series of papers at the forthcoming American Chemical Society meeting in Kansas City.

The applicability of various methods of analysis to the industrial sector was discussed, with special reference to the necessity of performing HRGC. It was conceded that LRGC was sufficient to analyze undifferentiated Aroclors, or other commercial PCB products, in various oil samples. The application of simple methods to PCB degradation products, or contaminants such as PCDFs, was said to be nonspecific. In terms of the discussion which suggested that LRGC was relatively inaccurate and insensitive as a means of measuring LPCB, it could be concluded that the use of HRGC might be essential in identification of PCBs incidentally produced during industrial processes, such as presented in the later session on incidentally produced PCBs in industrial processes.

CHAPTER 3

EXPOSURE STUDIES - ENVIRONMENTAL RESIDUES AND BIOACCUMULATION

The first paper in this chapter, encompassing production, distribution, and environmental fate of PCBs, offers data which estimates the total PCBs contamination of the mobile environment and demonstrates where the highest concentrations and largest quantities of the compound presently exist. Time-course studies are presented to determine the relative PCBs contaminations over a number of years. Qualitative analyses of individual isomers or homologs are performed to evaluate the compounds which are more likely to be environmentally or biologically degradable. Finally, environmental sampling data is presented which confirm the build-up of selected homologs.

The second paper extends these results to living organisms. Studies of fish and human populations reveal selective bioaccumulation of PCBs isomers and homologs which relate to their degree of chlorination, substitution in the para position, and occurrence of adjacent unsubstituted carbons. Problems associated with achieving PCBs steady-state levels in animals, followed by PCB elimination are extensively covered. Further discussed in detail is the effect of the reproductive cycle and lactation in fish and humans, respectively, on the elimination of PCBs body-burden.

The issues of the socio-medical aspects of lactation, the structure-activity relationships to metabolism, bioaccumulation, and toxicity, and the problems of measuring PCBs contamination in the Great Lakes are elaborated upon in the discussion summary.

DISTRIBUTION AND FATE OF CHLOROBIPHENYLS IN THE ENVIRONMENT

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INTRODUCTION

This paper reviews recent information on the input of PCB products and chlorobiphenyls to the environment, their distribution and fate, and on residues of these materials in environmental samples. The information is derived from estimates of chlorobiphenyl release, laboratory studies, and residue analysis. An understanding of the interrelation of these varied studies, and a projection to actual environmental behavior is extremely important to serve as a basis for control action for these compounds. More broadly, the chlorobiphenyls, along with several other materials for which there is an extensive database, serve as model compounds for predicting the behavior of the whole spectrum of synthetic and natural chemicals. It is, therefore, important that periodic updates be made of the whole picture regarding the environmental behavior of the chlorobiphenyls.

ENVIRONMENTAL ENTRY OF CHLOROBIPHENYLS

Polychlorinated biphenyls, products produced by the chlorination of biphenyl, were used extensively during 50s, 60s and early 70s. Their production and use were sharply curtailed, first voluntarily, and then by regulation during the 1970s. An estimated 610 x 10^6 kg PCBs were produced and used in the U.S. through 1975, of which an estimated 82 x 10^6 kg has been released to the mobile environment (NAS 1979). The remainder has either been destroyed, placed in landfills, or is still in use.

PCB products have not been manufactured in the U.S. since 1977. Chlorobiphenyls have been produced in small quantities as by-products of other manufacturing processes. Manufacturing exemptions have been filed with EPA for the production of products containing chlorobiphenyls in concentrations in excess of 50 ppm. Presumably, total quantities produced and the amount entering the biosphere could be estimated from this information. A recent survey of the chemical industry revealed 0.0062 x 10^6 kg of chlorobiphenyls are generated annually as incidental contaminants at concentrations below 50 ppm (Table 1). Less than 0.0004 x 10^6 kg of this amount annually enters the mobile environment.

The major current loss of PCBs is believed to be leakage from capacitors, transformers, and other electrical equipment in use. An upper bound estimate of such entry into the environment provided by EPA is about 0.18 x 10^6 kg annually (47 FR 17425, April 22, 1982).

Chlorobiphenyls are also produced as by-products of high temperature combustion processes (Richard and Junk 1981, Junk and Ford 1980, Vick et al. 1978). These include municipal and industrial incinerators, coal-fired electric power plants, and the burning of wood. The origin of the chloro-

Table 1. Incidental Chlorobiphenyl Production and Disposal

Total Annual Production (kg)		6,200
Disposal		
Incineration		4,100
Salt Water Discharge		800
Landfill		700
Underground Injection		300
Enclosed Solid Products		200
Other Products		100
	TOTAL	6,200

Chemical Manufacturer Association, November 15, 1981.

biphenyls is not believed to be the combustion of materials containing PCB, since addition of refuse containing PCB to a coal fired burner did not increase the amount of chlorobiphenyls in the effluents. The magnitude of chlorobiphenyl formation from this source is unknown. The amounts reported in effluent gases range from 0.0007 $\mu g/m^3$ to 200 $\mu g/m^3$ compared to ambient air levels of 0.0001 to 0.01 $\mu g/m^3$. A review of organic emissions from combustion processes has been provided by Junk and Ford (1980).

Still another source of PCB to the mobile environment is leakage from landfills and equipment dumps. An estimated 140×10^6 kg PCB had been disposed of in landfills and equipment dumps through 1978 (NAS 1979). Very little data is available for estimating the quantity of PCB entering the mobile environment from this source. The amount leaking into groundwater is believed to be negligible, but small amounts are believed to volatilize from relatively exposed sites (NAS 1979).

DISTRIBUTION AND FATE OF CHLOROBIPHENYLS

The distribution and fate of chlorobiphenyls depends on their release patterns, partitioning and dispersion, and their susceptibility to degradation by various routes. The chlorobiphenyls that constituted PCB products were released over a period of years as a result of a wide variety of uses. Load estimates in the atmosphere, hydrosphere and lithosphere for U.S. manufactured PCBs that have entered the mobile environment are shown in Table 2 (NAS 1979). The percentages are based on an input of 82×10^6 kg from U.S. production and release. The major reservoir for PCB is reported to be the Atlantic Ocean. The atmosphere is a very minor reservoir, but is believed to have served as a major transport vehicle for PCB. Freshwater reservoirs do not contain a large fraction of the total load, but the highest concentrations are usually found in freshwater ecosystems.

Much attention has been focused on the distribution of PCBs in the Great Lakes. A recent summary has been provided by Eisenreich (1982). He concludes the present PCB burden for the entire Great Lakes is 0.44 to 0.5×10^6 kg of which 88% is in the sediments. Estimates for each lake are shown in Table 3. Eisenreich concluded that the major current input to the Upper Lakes is via the atmosphere, and that input is nearly balanced by losses due to burial in the sediment.

Once released, the PCB products exposed to the atmospheric or aquatic environments were slowly vaporized or dissolved. Further distribution and/or degradation took place depending on the chemical, physical, and biological properties of the individual compounds. These properties vary considerably within the family of chlorobiphenyls, and they greatly influence the ultimate fate of each compound. Thus, the properties of the individual chemicals will be discussed in order to understand their fate and the observations reported on residues of these materials.

PHYSICAL PROPERTIES

The physical properties of the chlorobiphenyls have recently been reported or summarized (NAS 1979, Mackay et al. 1980, Banerjee et al. 1980, Westcott et al. 1981, Neely 1983, McDonald, unpublished, Smith et al. 1964).

Table 2. Environmental Load Estimates of PCB for the U.S. and the Atlantic Ocean[a]

	Amount x 10^5 kg	Percentage Based on Total Release
Atmosphere	0.018	0.02
Hydrosphere		
Freshwater	0.012 - 0.035	0.01 - 0.04
Freshwater sediment	1.4 - 7.1	1.7 - 8.6
Freshwater biota	0.03	0.04
Marine water	6 - 66	7.3 - 73
Marine sediment	0.66 - 2.7	0.7 - 3.3
Marine biota	0.03	0.04
Lithosphere	0.14 - 2.8	0.2 - 3.4

[a]NAS 1979.

Table 3. PCB Burdens in the Great Lakes[a]

Lake	PCB Amount x 10^3 kg
Superior	38
Michigan	57
Huron	69
Ontario	122
Erie	187

[a]Eisenreich 1982.

Important properties are shown in Table 4. Solubility and volatility generally decrease with increased degree of chlorination, although individual isomers may show some variation.

From these properties it is possible to calculate distribution coefficients (Dilling 1979, Karickhoff et al. 1979, Chiou et al. 1977, Veith et al. 1979) for individual congeners between various media as shown in Table 5. Such information is essential for predicting what part of the environment will contain residues of the compounds, or whether the compounds will get to regions where degradation will occur. An inspection of the properties and distribution coefficients clearly shows increasing levels of chlorination increases the tendency for adsorption to soils and for bioconcentration. Low degrees of chlorination increase the mobility of the compound, both into the atmosphere and into water, and increase the probability for undergoing degradation.

ENVIRONMENTAL DEGRADABILITY

Several studies have been reported describing the biodegradability of chlorobiphenyl congeners (Bailey et al. 1981, Ahmed and Focht 1973, Anderson 1980, Furukawa et al. 1978, Wong and Kaiser 1975, Reichardt et al. 1981). All present a consistent picture. Chlorobiphenyls with a low degree of chlorination are readily biodegradable, while the highly chlorinated congeners are increasingly resistant to biodegradation.

The work of Bailey *et al.* (1981) is particularly noteworthy since ^{14}C-labeled compounds were used and good material balances were obtained in his studies. Degradation studies were carried out in river water. The pattern of degradation observed for 2-chlorobiphenyl is shown in Figure 1. The parent chlorobiphenyl degraded rapidly ($t_{1/2}$, approximately 2 days), one isolatable intermediate degradation product was observed (chlorobenzoic acid), which rapidly degraded to $^{14}CO_2$ and presumably water and inorganic chloride. These observations are consistent with the early work of Ahmed and Focht (1973) who proposed the degradation pathway shown in Figure 2, and also the later work of Anderson (1980), who observed chlorobenzoic acids as degradation intermediates. The half-life for degradation was similar for concentrations ranging from 1 to 100 µg/L for all three isomers of monochlorobiphenyl. By way of contrast, the tetra-substituted compound, 2,2',4,4'-chlorobiphenyl, studied under similar conditions showed no measurable degradation during a period of 30 days. Anderson (1980) found the degradability of chlorobiphenyls in lake sediments decreased with increasing degree of chlorination, with half-lives ranging from a few days for dichlorobiphenyl to 200 days for pentachloro isomers. Biodegradability is also affected by the position of chlorine substitution on the biphenyl ring, particularly for poly substituted derivatives. No degradation was observed under anaerobic conditions for any of the chlorobiphenyls.

Studies of the atmospheric degradation of chlorobiphenyls are sparse. Appleby (1976) reported that 2-chlorobiphenyl degraded under simulated atmospheric conditions with a half-life of a few days. Theoretical work by Hendry (1979) is consistent with the work of Appleby, and suggests the more highly chlorinated congeners are more resistant to atmospheric photodegradation. Recent more definitive work by Dilling (1983) confirmed the work of Appleby.

Table 4. Chlorobiphenyl Physical Properties[a]

Chemical	Water Solubility umole/L, 25° C	Vapor Pressure mm Hg, 25° C
2-CBP	26	8.4×10^{-3}
4-CBP	12	4.6×10^{-3}
2,2'-CBP	4.9	2.1×10^{-4}
4,4'-CBP	0.3	1.9×10^{-5}
2,5,2'-CBP	3.9	9×10^{-5}
2,3,4'-CBP	0.3	
2,5,2',5'-CBP	0.09	3.7×10^{-5}
3,4,3,4'-CBP	0.3	
2,4,2',4'-CBP	0.23	8.6×10^{-5}
2,3,4,5,2'-CBP	0.028	

[a]NAS 1979, Mackay et al. 1980, Banerjee et al. 1980, Westcott et al. 1981, Neely (1983), McDonald (unpublished data), Smith et al. 1964.

Table 5. Environmental Distribution Coefficients

Compound	Distribution Coefficients		
	Air/Water H[a]	Solid/Water K_{sw}[b]	Fish/Water BCF[c]
2-CBP	0.017	660	2,000
4-CBP	0.020	270	960
2,2'-CBP	0.002	1,100	3,300
4,4'-CBP	0.004	700	2,100
2,5,2'-CBP	0.003	5,300	12,000
2,5,2',5'-CBP	0.023	16,000	31,000
2,4,2',4'-CBP	0.020	35,000	60,000
2,3,4,5,2'-CBP		29,000	52,000

[a] In units of $\frac{\text{mg/L in air}}{\text{mg/L in water}}$, calculated from Dilling 1979.

[b] Assumes 2% organic carbon, calculated from Banerjee et al. 1980 and Karickhoff et al. 1979.

[c] Calculated from Banerjee et al. 1980 and Veith et al. 1979.

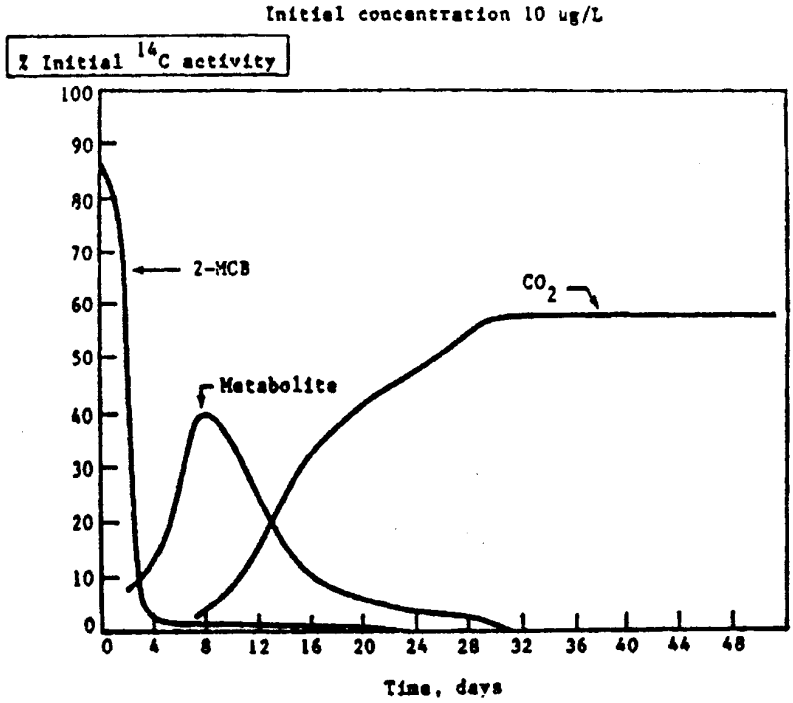

Figure 1. Biodegradation of 2-monochlorobiphenyl.

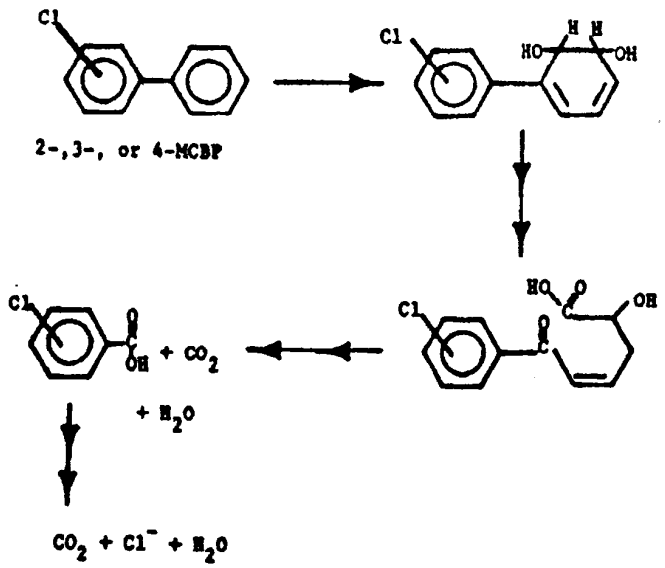

Figure 2. Biodegradation pathway of monochlorobiphenyls.

He studied the photodegradation of all three monochlorobiphenyl isomers in a glass chamber using benzene and a stable chlorofluorocarbon as internal standards. The study was carried out with two different surface to volume ratios, showing that the reaction was in the gas phase and not surface catalyzed. The results are illustrated in Figure 3 showing the disappearance of 2-chlorobiphenyl under dark and light conditions. By comparing the rates of disappearance of each isomer with that of benzene under light and dark conditions, and knowing the rate constant for OH radical attack on benzene (Perry and Pitts 1977) from previous published work, Dilling was able to calculate corresponding rate constants and average tropospheric halflives for the three monochlorobiphenyl isomers. These are shown in Table 6. All three monochlorobiphenyl isomers degrade under simulated atmospheric conditions with half-lives of about one day.

PREDICTION OF ENVIRONMENTAL RESIDUES

The laboratory data shows that increasing levels of chlorine substitution on biphenyl results in a greater tendency for adsorption to solids, an increase in the bioconcentration potential, and a greater degree of persistence. On a qualitative basis it is clear that monochlorobiphenyl is likely to disperse and degrade rapidly, while the highly chlorinated compounds (especially tetra and above) are likely to be persistent and appear as environmental residues. Neely (1981) recently integrated this data into a mathematical model intended to simulate the "average" world in order to predict the lifetime of selected chlorobiphenyl congeners in the environment. His conclusions, shown in Figure 4, predict a half-life of about 3 days, for monochlorobiphenyls, 150 days for di, 579 days for tri, 1044 days for tetra, and 3445 days for penta. If atmospheric degradation is included for monochlorobiphenyls, the half-life is reduced to one day. If conditions of temperature, microorganism concentration, etc. differ markedly from those assumed by Neely, differences in persistence would be expected. However, the relative degradation rates between different congeners should remain nearly constant.

Neely (1983) also modeled the situation in Lake Michigan with respect to homolog distribution. Shown in Figure 5 are the differences expected between an assumed original "PCB fluid" that may have entered the lake, and the distribution of chlorobiphenyls expected in both water and fish. Mono and di go from 1 and 16% of the chlorobiphenyls in the original fluid to about 0 and 6.4% in water and 0 and 2% in fish, respectively. On the other hand, penta goes from 10% of the chlorobiphenyls in the fluid to 10% in the water and 48% in the fish. The differences reflect preferential biodegradation of the mono and di chloro compounds based on laboratory studies of their biodegradability, and also the greater tendency for bioconcentration for the penta.

ENVIRONMENTAL RESIDUES

Reports describing the homolog distribution of chlorobiphenyls found in the environment are consistent with Neely's predictions. Figure 6 shows the distribution of homologs sold in the U.S. during the period 1930-1978. Trichlorobiphenyl was the most abundant homolog of the series, followed by tetra, penta, and di.

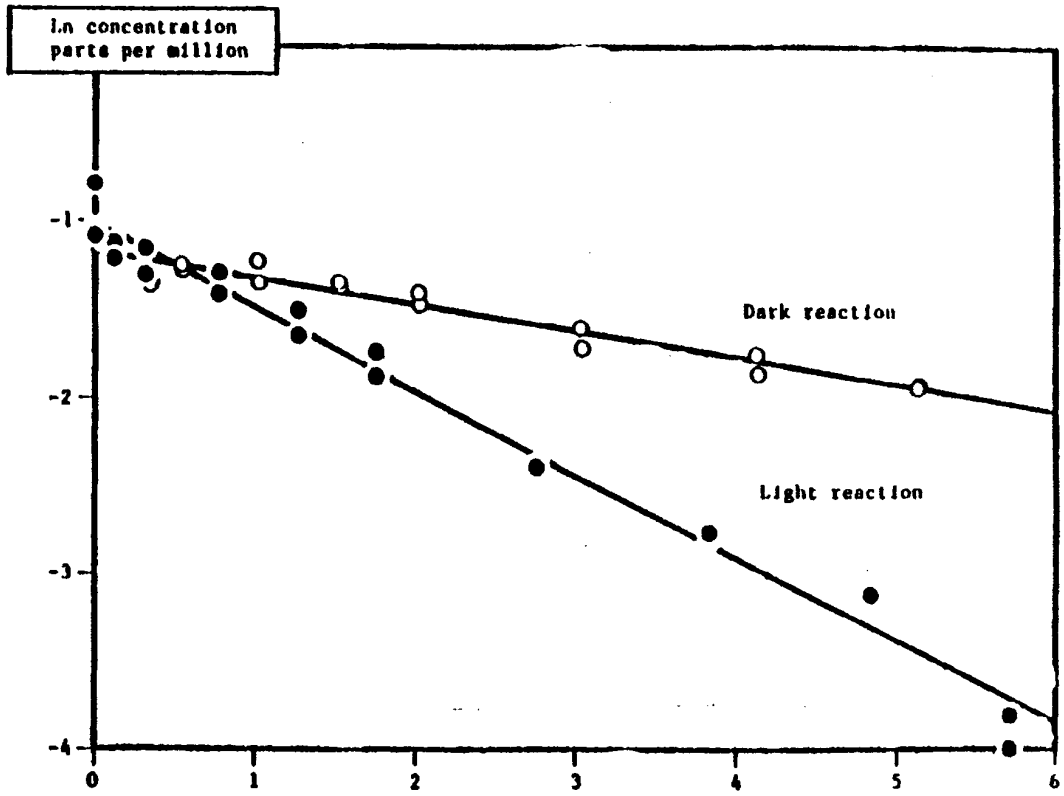

Figure 3. Simulated atmospheric degradation of 2-chlorobiphenyl

Table 6. Atmospheric Degradation Parameters for Monochlorobiphenyls[a]

	$k_{\cdot OH}$	$t_{1/2}$ (Tropospheric Average)
2-CB	$5.4 \times 10\ cm^{-12}\ cm^{3}\ molecule^{-1}\ sec^{-1}$	1.5 day
3-CB	1.0×10^{-11}	0.77
4-CB	1.2×10^{-11}	0.68

Assumes $(\cdot OH)ave = 10^{6}\ cm^{-3}$

[a]Dilling et al. 1983.

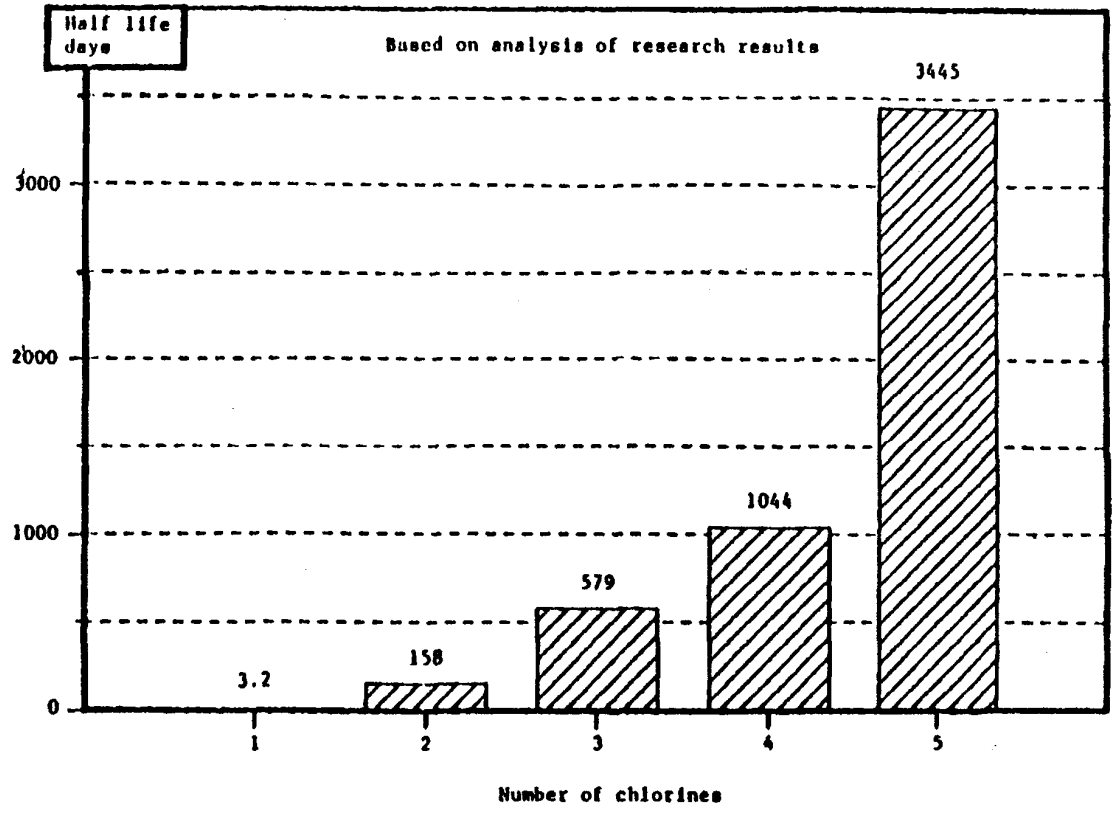

Figure 4. Environmental persistence of selected chlorinated biphenyl isomers.

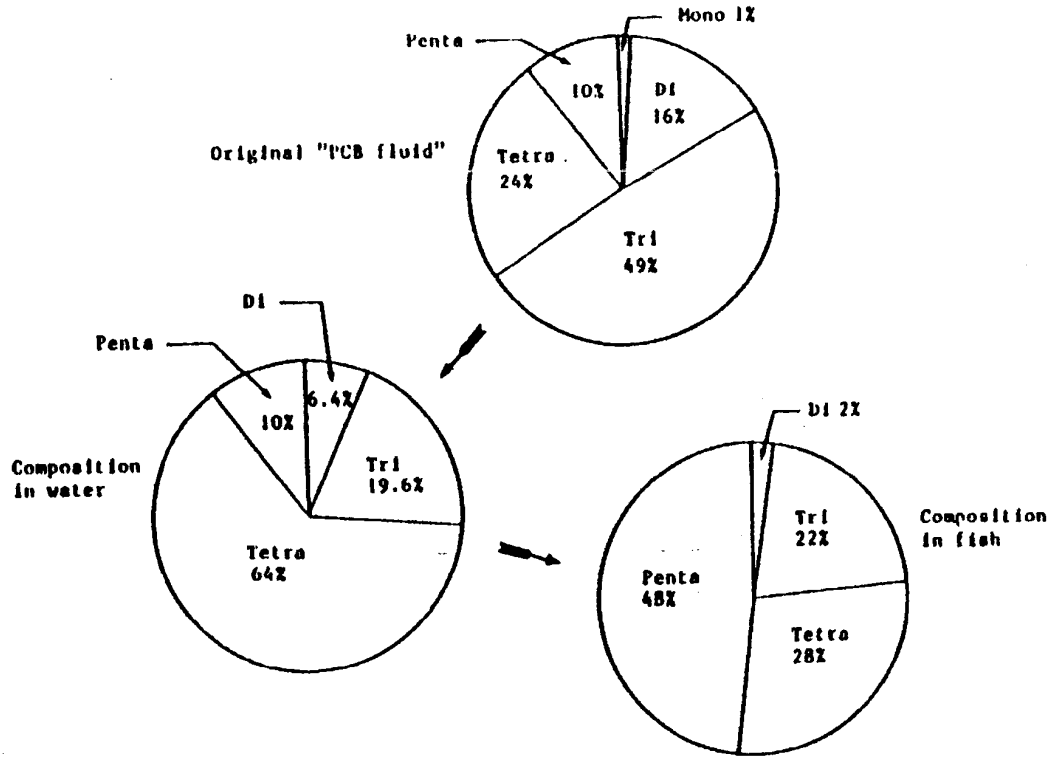

Figure 5. Predicted homolog distribution of chlorobiphenyls in Lake Michigan.

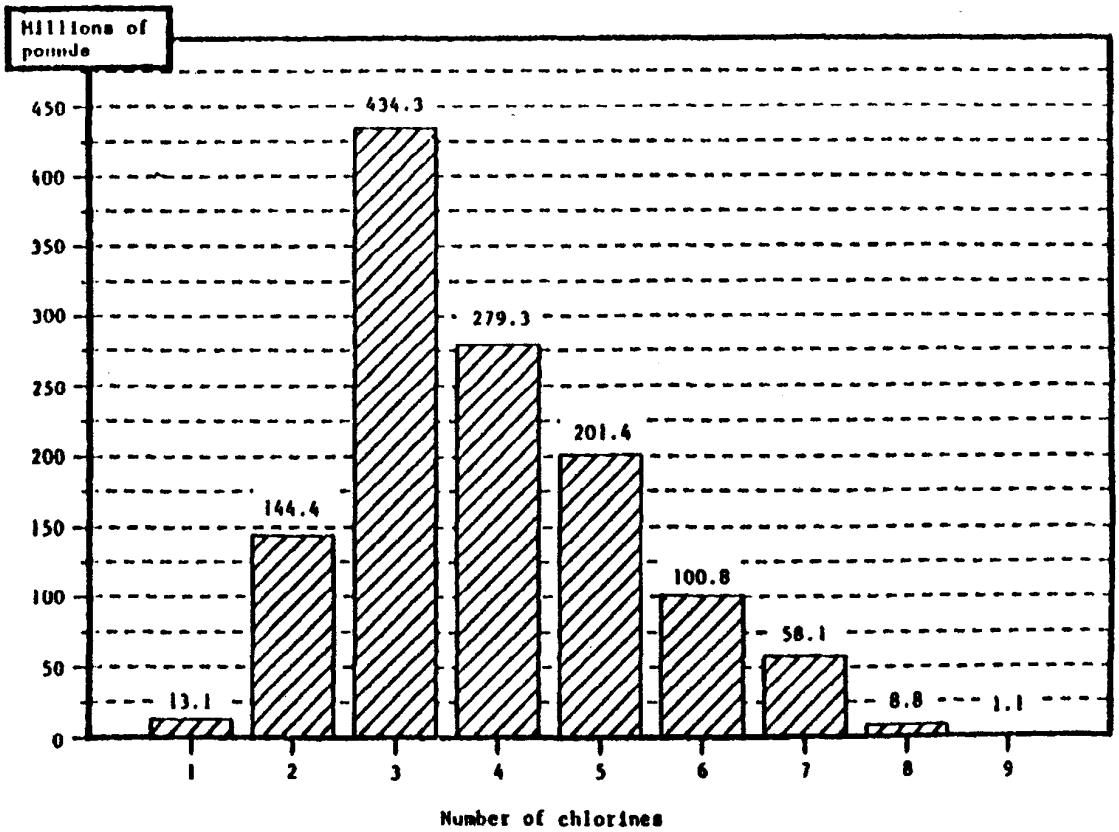

Figure 6. Chlorinated biphenyls sold in the U.S. 1930 - 1978.

Chlorobiphenyls found in plasma and adipose tissue of capacitor manufacturer workers (Figure 7), who were exposed to PCB products with homolog distribution as manufactured, showed a shift in homolog distribution to the more highly chlorinated compounds (Wolff et al. 1981). Tetra was the predominant chlorobiphenyl found, followed by tri, penta and hexa. Di was reduced to very low levels, while mono was not observed. The shift in distribution can be explained by the fact that chlorobiphenyls with a low degree of chlorination, especially mono and di, are cleared rapidly from mammals, Figure 8 (Kaley et al. 1976, Matthews and Anderson 1975). Thus, accumulation favors the highly chlorinated compounds.

The additional effect of the environment on the homolog distribution in human tissue can be seen by examining Figures 9 and 10 (Jensen and Sundstrom 1974, Mes et al. 1980). These data were from humans exposed to chlorobiphenyls in the general environment rather than in the workplace. The homolog distribution is shifted to even higher degrees of chlorination, with hexa and hepta levels predominating. The disappearance of dichlorobiphenyls and the dramatic reduction of trichlorobiphenyls indicate that transport and degradation in the environment has greatly reduced the levels of these compounds. Thus, both the environment and mammalian organisms tend to preferentially degrade or eliminate biphenyls with a low degree of chlorination compared to those with a high degree of chlorination.

The preferential degradation and excretion of lower chlorinated biphenyls is probably a key reason why they exhibit less potential for chronic toxicity than that shown by PCB products or highly chlorinated biphenyls. In a 31-day gavage toxicity study in rats, approximately 100 times more monochlorobiphenyl was required to produce liver effects equivalent to those noted for Aroclor 1254 (Dietz et al. 1982). Continuous flow studies (32 days) with fishes also showed 2-chlorobiphenyl was less toxic than 2,2',4,4'-tetrachlorobiphenyl by a factor of about 30 (Dill et al. 1982).

The overall levels of PCB in Lake Michigan are declining (Hartig 1981). Figure 11 shows levels of PCB in chubs taken from Lake Michigan. The PCB concentration has dropped from 5.5 ppm in 1974 to 2.2 ppm in 1980. Figure 12 shows PCB levels in lake trout and coho salmon are also dropping, although there are no data taken since 1976. Figure 13 indicates a dramatic drop in PCB levels in gull eggs taken from islands in Lake Michigan. The drop in PCB levels in gull eggs closely parallels the drop in PCB levels in chubs.

Eisenreich (1982) concluded the residence times of PCB in Lake Superior and Lake Michigan waters are 2 to 7 years and 1.5 to 4 years, respectively. Residence times in these lake waters depend in part on the rate of permanent burial of these compounds in sediments. Once buried, the PCB is not accessible to water organisms, and does not accumulate in the food chain. No data has been reported on the degradation of PCB in the actual sediments in the lakes. Although, as discussed earlier, sediments taken into the laboratory have shown degradation of chlorobiphenyls (Anderson 1980).

Atmospheric levels of PCB over Lake Superior, based on limited sampling shown in Table 7, are also decreasing (Eisenreich, Personal communication). Reported levels have dropped from a range of 0.9 to 3.5 ng/m^3 in 1978 to 0.1 to 0.6 ng/m^3 in 1981. It is possible to estimate yearly production and

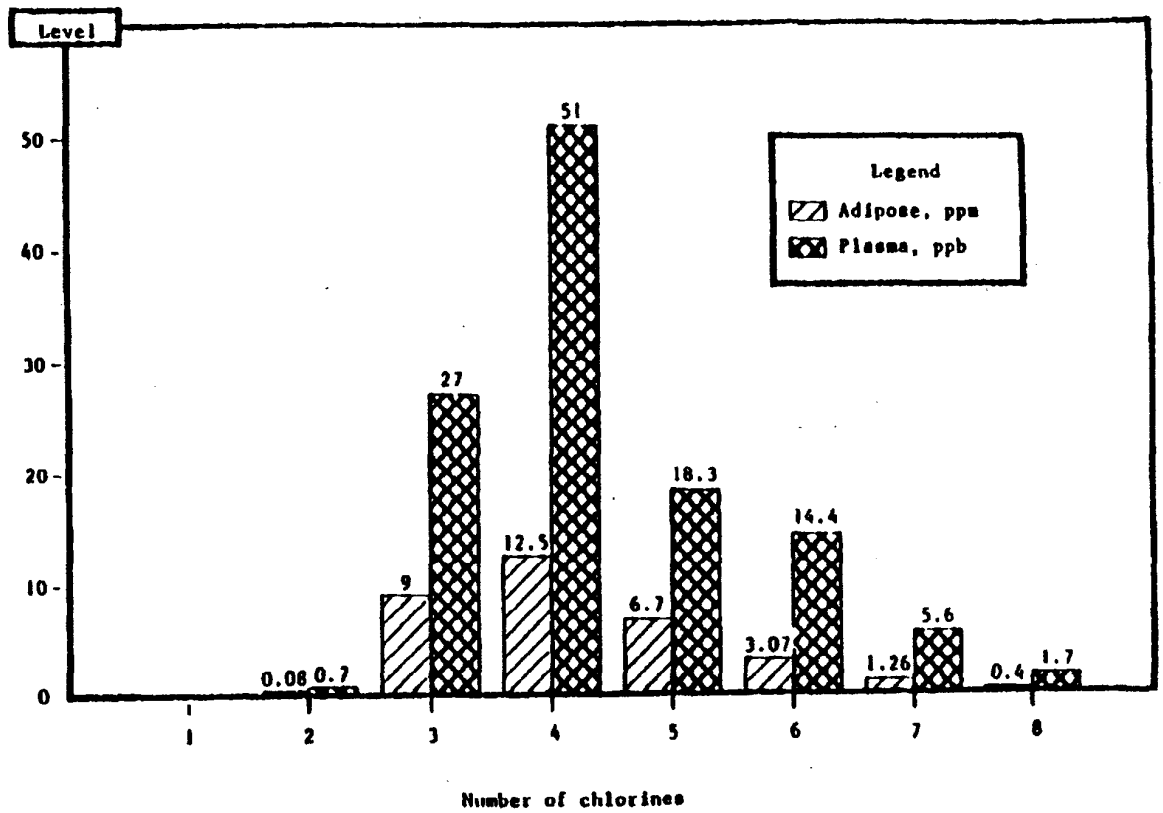

Figure 7. PCB levels in capacitor manufacturer workers.

**Note: Elimination of hexachlor from fat was not observed.

Figure 8. Bioclearance of chlorinated biphenyls from rats.

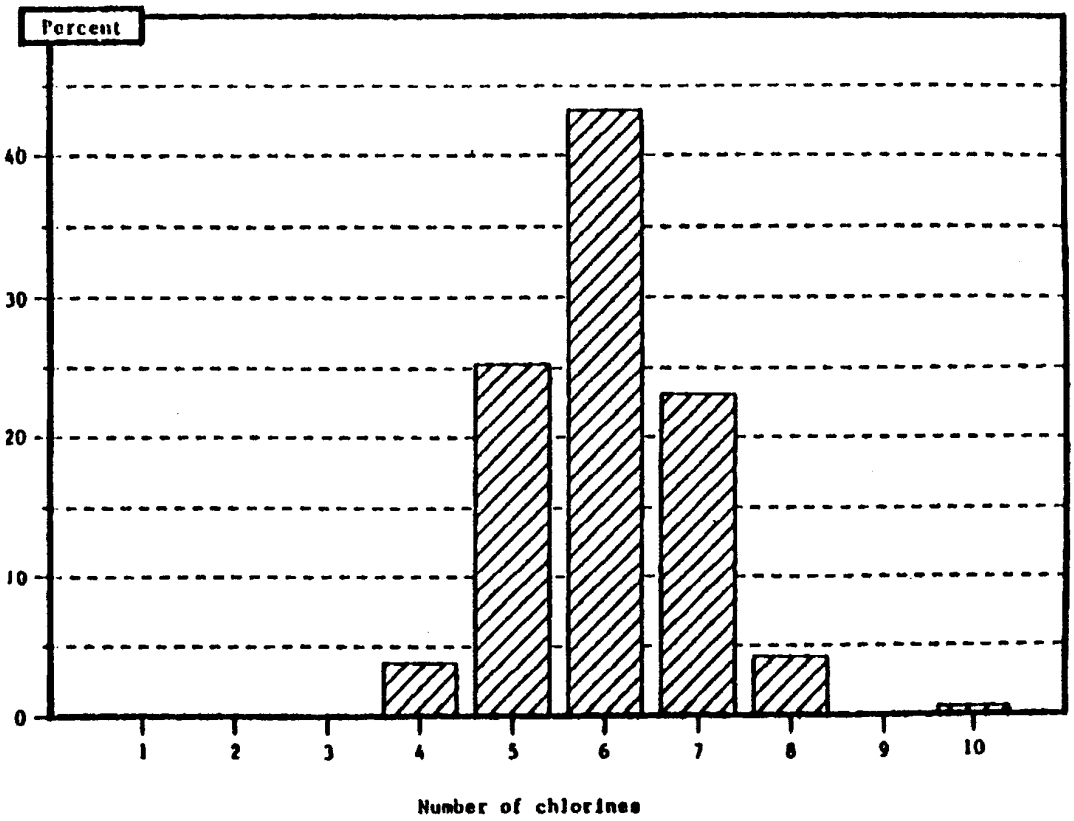

Figure 9. Distribution of chlorinated biphenyls found in selected human fat samples.

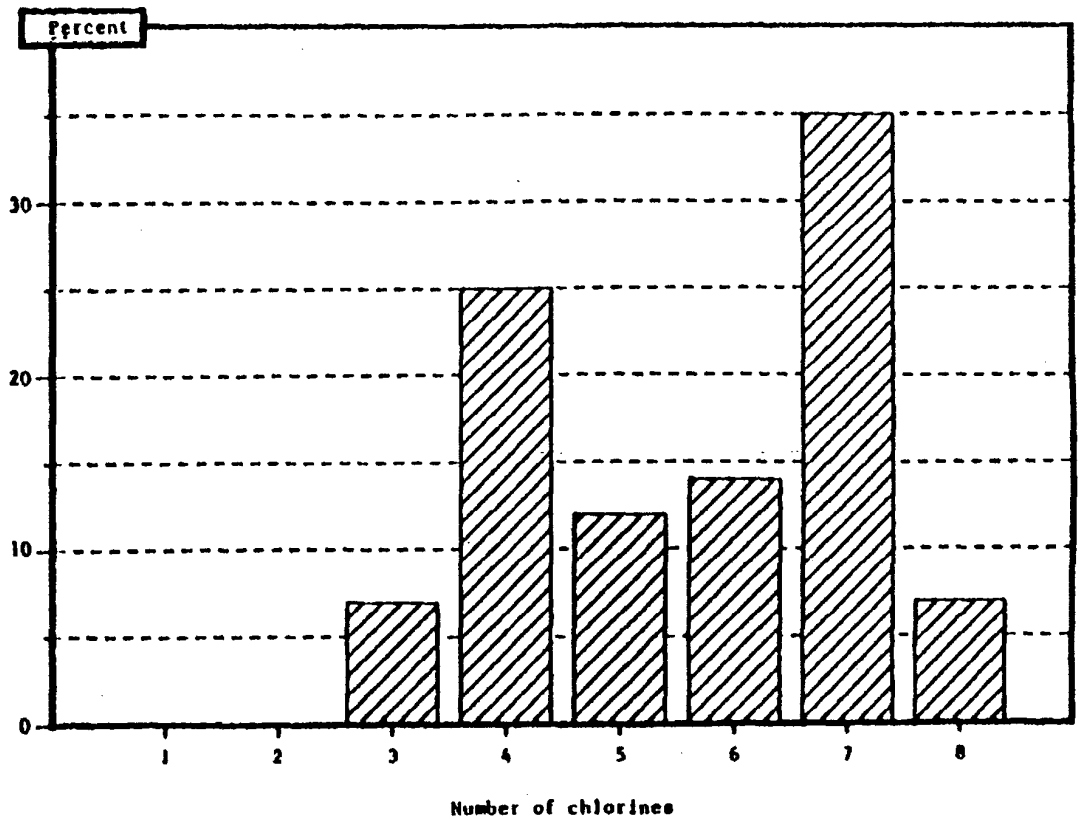

Figure 10. Distribution of chlorinated biphenyls found in human milk.

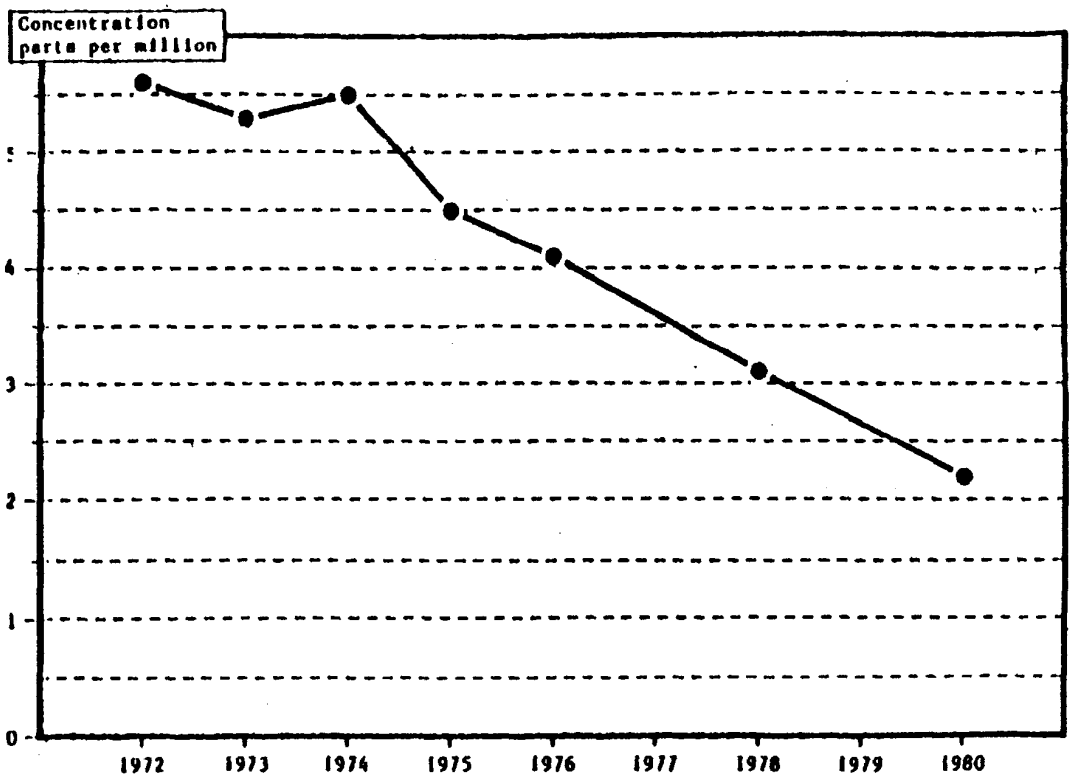

Figure 11. Chlorinated biphenyl levels in Lake Michigan chub.

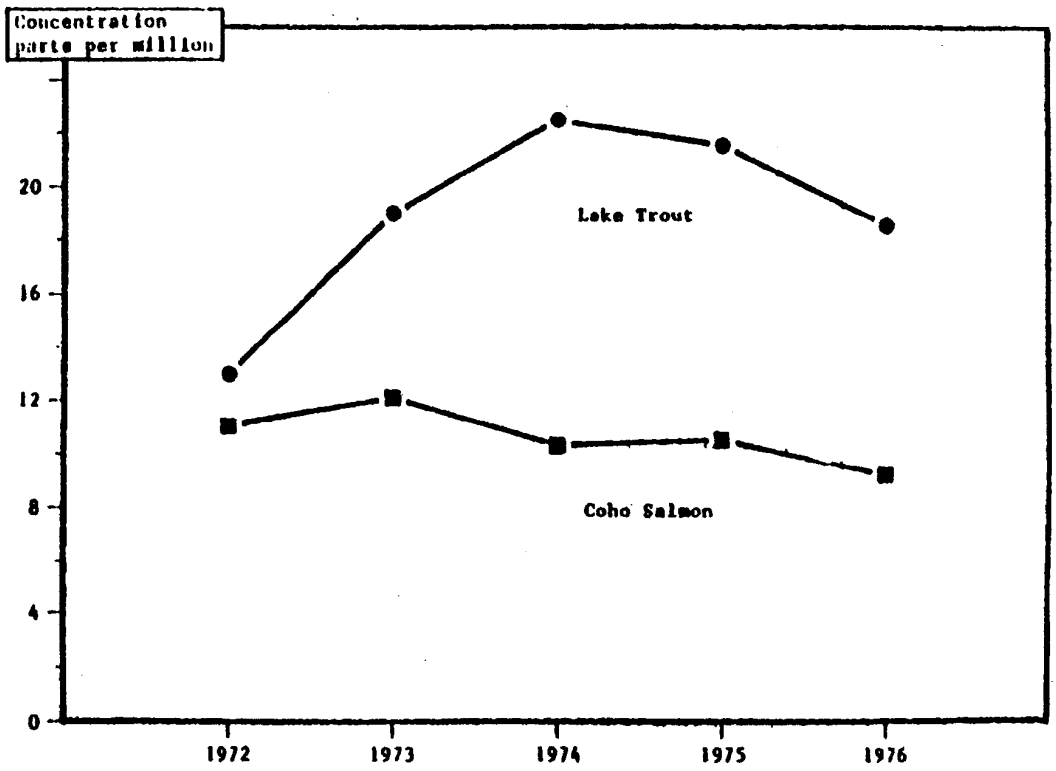

Figure 12. Chlorinated biphenyl levels in Lake Michigan sport fish.

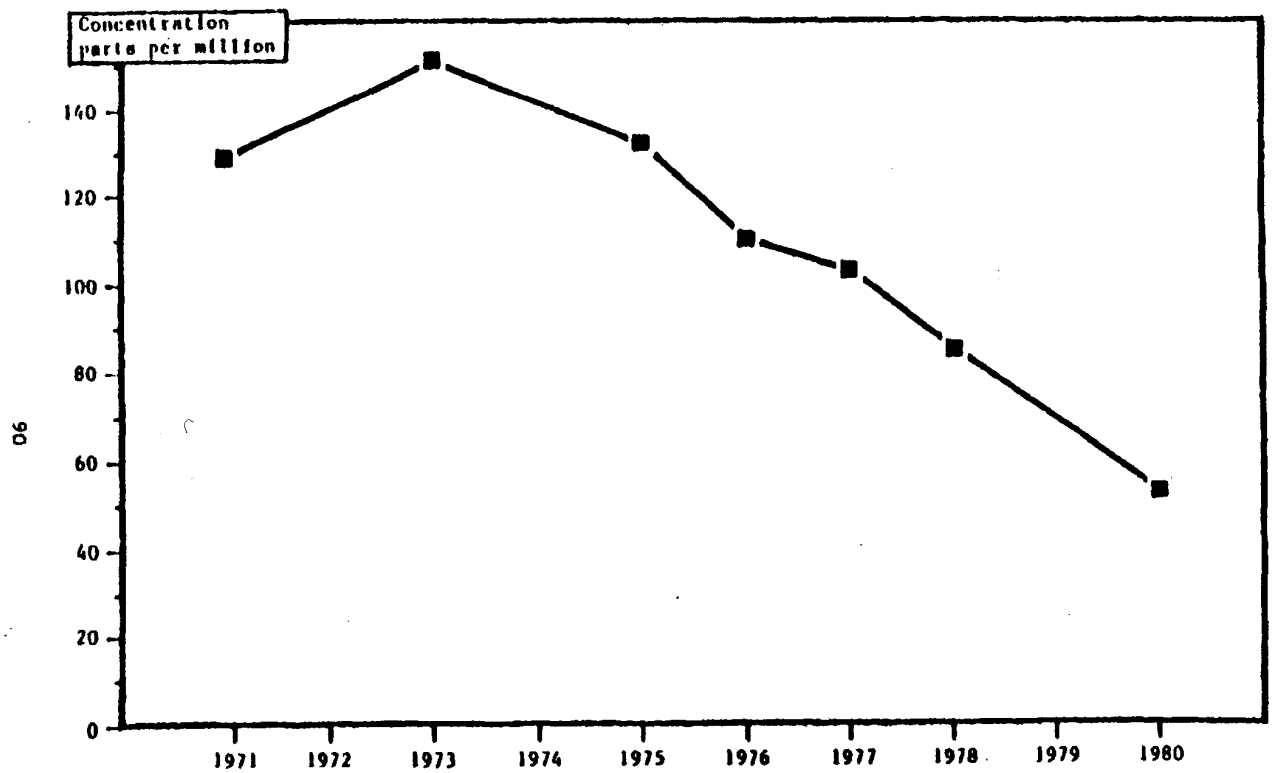

Figure 13. Chlorinated biphenyl levels in gull eggs on Lake Michigan islands.

Table 7. PCB Concentrations in Air Over Lake Superior[a]

Year	Mean	Range, ng m^{-3}	S.D.	n
1978	1.5	0.9 - 3.5	0.8	13
1979	0.9	0.4 - 1.4	0.4	8
1980	1.0	0.1 - 2.5	1.0	8
1981	0.3	0.1 - 0.6	0.1	9

[a]Eisenreich, personal communication.

release of PCB in the U.S. from the information reviewed in this paper. The data are presented graphically in Figure 14a. Production of PCB dropped drastically during the early 70s and intentional production of the mixtures ended in 1978. A small incidental production (<0.3 million lbs/yr) has continued into the 1980s. Release of PCB to the mobile environment is estimated at 5 to 12 million lbs/yr during the 10 year period 1962-1972. Since 1978 the release has been <0.5 million lbs per year.

Data on the levels of PCB in Lakes Michigan and Superior, as summarized in Figure 14b, reflect the response of these lakes to the reduction in release of the compounds to the mobile environment. Levels began to drop about one year after the release rate was reduced, and have shown a steady decline since that time. Similar trends have been observed for DDT and mercury in the Great Lakes (Hartig 1981). Further reduction with time is expected as removal of these compounds from the mobile environment through degredation and removal by burial continues.

Conclusions

1. Since production of PCB began, an estimated 80 to 90 x 10^6 kg have been released to the mobile environment in the U.S.

2. PCB products and chlorobiphenyls still enter the mobile environment in small amounts (approximately 0.2 x 10^6 kg/yr).

3. The major reservoir for PCB released in the U.S. is the North Atlantic Ocean, but freshwater systems contain the highest concentrations.

4. PCB levels in the Great Lakes (air, water, and fish) have dropped dramatically since releases were curtailed in the mid-70s. PCB concentrations in Lake Michigan chubs have dropped from 5.5 mg/kg in 1974 to 2.2 mg/kg in 1980.

5. Laboratory studies have shown that the degradability of chlorobiphenyls decreases with increasing chlorine content. For example, monochlorobiphenyl degrades readily in lake sediments, natural waters and air with half-lives of a few days or less. However, the half-lives of tetra and higher homologs is of the order of months to years.

6. Residues measured in human tissue show no monochlorobiphenyl, very little dichlorobiphenyl and reduced trichlorobiphenyl compared to the homolog distribution in PCB products manufactured in the U.S. This observation tends to confirm the laboratory observations on the relative degradability of the lower chlorinated biphenyls compared to the highly chlorinated compounds.

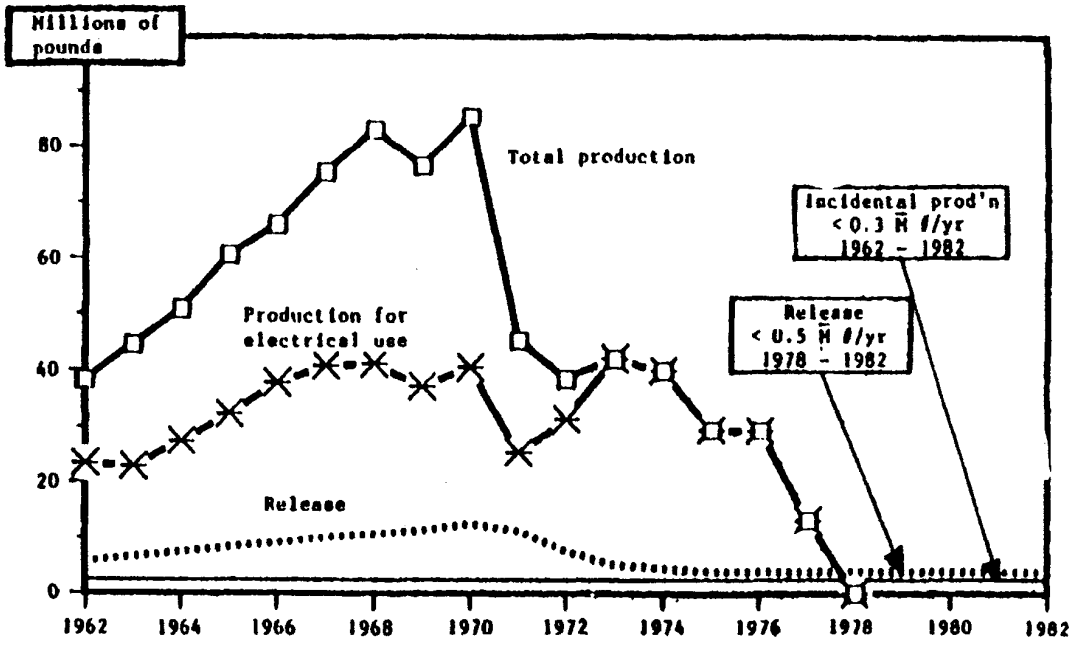

Figure 14a. PCBs - Annual U.S. production and estimated release to the environment.

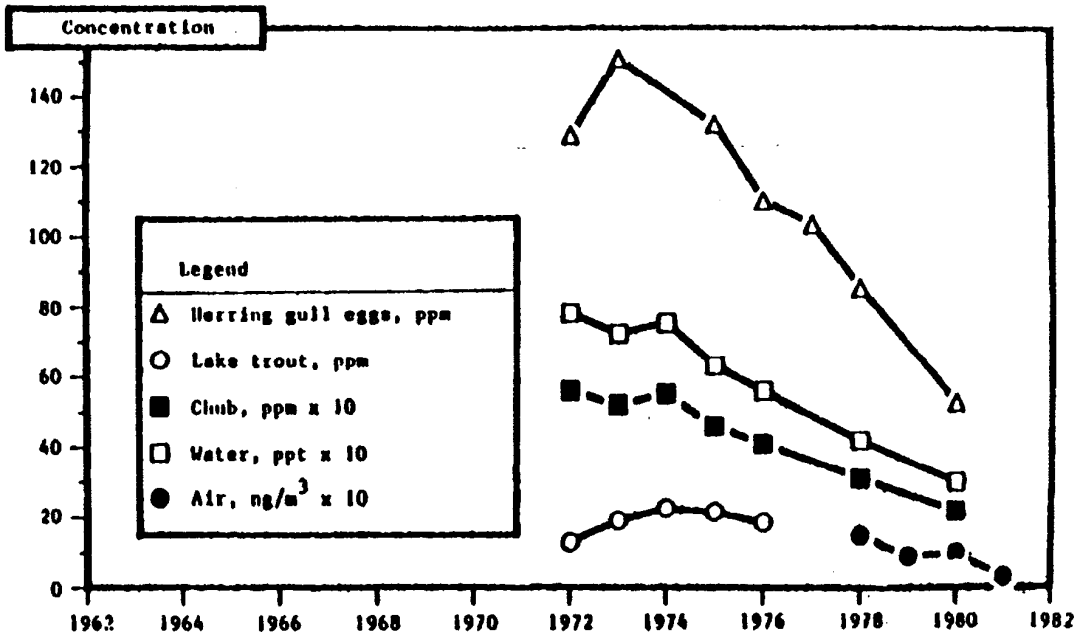

Figure 14b. PCBs in the Great Lakes environment.

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FACTORS AFFECTING THE BIOACCUMULATION AND PERSISTENCE OF POLYCHLORINATED BIPHENYLS

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Polychlorinated biphenyls (PCBs) are widespread persistent industrial contaminants in the global ecosystem. Originally used in paints, inks, hydraulic fluids and electrical components, PCBs have been found in the atmosphere, fresh and salt water, soil and tissues of numerous animal species. Because of their high lipid solubility, PCBs tend to accumulate in adipose tissue and thus become biomagnified through the food chain. Although numerous investigators have examined the pharmacokinetic behavior of PCBs in mammalian species, there have been relatively few studies which consider these phenomena in the lower vertebrate forms. This paper attempts to compare the accumulation, distribution, persistence and metabolism of polychlorinated biphenyls in mammals and aquatic species.

BIOACCUMULATION

As is the case in the higher animals, fish are exposed to environmental contaminants primarily through the diet and inhalation (via the gill). Whether a particular chemical is absorbed and accumulated by aquatic species is dependent to a great extent on its physico-chemical characteristics, most notably, its water solubility and lipophilicity. PCBs have been shown to bioaccumulate in fish species and aside from its obvious significance from an ecological standpoint, bioaccumulation among lower vertebrates has been recognized as a potential human health hazard as well.

Bioaccumulation can be assessed by the calculation of the bioaccumulation factor (concentration of chemical in whole fish/concentration of chemical in water). This factor is a characteristic of the fish species and duration of exposure to the compound and is independent of the water concentration of the chemical. Figure 1 illustrates the time dependency and magnitude of bioaccumulation of Aroclors 1254 and 1248 by channel catfish exposed to these mixtures in water (Mayer et al. 1977). It can be seen that even after 77 days of exposure, the concentration of PCBs in fish had not reached the steady state even though bioaccumulation had proceeded to over 60,000 times the concentration of these mixtures in the exposure water. These data demonstrate that in order to achieve a steady state concentration of PCBs in fish, an extended duration of exposure is required. Steady state concentrations of PCBs were reached in fathead minnows which had been exposed to Aroclors 1242, 1248, 1254 or 1260 for a period of eight months (42 FR 6532, 1977). Bioaccumulation factors for individual minnows ranged from 32,000 for Aroclor 1242 to 307,000 for Aroclor 1254.

The bioaccumulation of PCBs in fish has also been assessed by the use of feeding studies. When coho salmon were fed diets containing increasing concentrations of Aroclor 1254, it was found that the higher the concentration

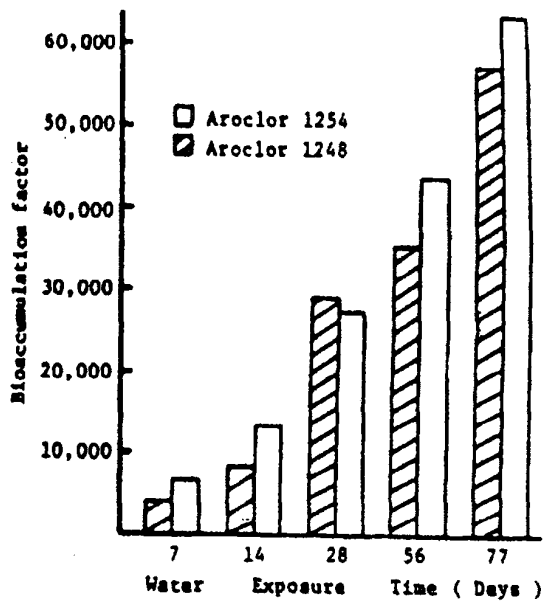

Figure 1. Bioaccumulation of Aroclors 1248 (5.8 µg/L) and 1254 (2.4 µg/L) in channel catfish following water exposure.

of the PCB in the diet, the longer it took for whole body PCB concentrations to reach a steady state (Mayer et al. 1977). Furthermore, the higher the concentration of Aroclor 1254 in the diet, the higher the steady state concentration in the animal. These data concerning time to steady state with different concentrations of PCBs may explain the results of Leatherland et al. (1979) who showed that PCB levels in coho salmon fed a high PCB diet (500 mg/kg) were only 3.2-fold higher than in coho fed a low PCB diet (50 mg/kg).

It is important to note that data concerning bioaccumulation of chemicals, including the PCBs, can be expressed as an absolute amount of chemical in the body at any given time or as the concentration of that particular chemical in a given tissue. This is an important concept since in long term exposure studies the fish are growing and one must deal with the combination of constant intake of chemicals and the tendency toward dilution of their concentration in the animal by growth. This is illustrated in Figure 2 where rainbow trout were fed 15 parts per million (ppm) of Aroclor 1254 for 32 weeks (Lieb et al. 1974). Graph A represents the PCB concentration in fish in ppm, B represents the total PCB content of the fish in micrograms, and graph C illustrates the body weight measured over the exposure period. It is clear that although the concentration in the fish began to level off at approximately 24 weeks of dietary exposure, the absolute amount of PCBs in the animals continued to increase. These data illustrate that although a steady state may be reached in terms of concentration in the animal during the exposure period, total PCB content in absolute micrograms/animal may continue to increase. When rainbow trout were fed a diet containing 15 ppm of Aroclor 1254 for 16 weeks and then were switched to a control diet, the fish which continued to receive PCBs continued to accumulate them, but the trout removed from the PCB-containing diet demonstrated no decrease in PCB content (Figure 3). It is important to recognize that the absolute amount of PCBs expressed as mg/fish did not change significantly from the 16th to 32nd week on the control diet. These data illustrate the lack of elimination of Aroclor 1254 even when animals were fed a control diet. If the data are expressed on a concentration basis however, the situation is quite different. This is illustrated in Figure 4 where PCB concentration in lipid is plotted against average fish weight and the time in weeks after removal from the PCB-containing diet. These data clearly show that the PCB concentration decreased over time after removal of the fish from the PCB-containing diet and this decrease in concentration can be accounted for almost entirely on the basis of dilution through weight gain of the animals.

An important factor which determines the extent to which PCBs are accumulated by fish is the degree of chlorination of the biphenyl nucleus. Generally, lower chlorinated PCB mixtures are accumulated to a lesser extent than more highly chlorinated mixtures. This is illustrated in Figure 5 by channel catfish accumulating Aroclors 1232 and 1248 (32 and 48% chlorine, respectively) to a lesser extent than 1254 and 1260 (54 and 60% chlorine, respectively) (Mayer et al. 1977). The lower accumulation is due to more rapid metabolism and elimination of the lower chlorinated chlorobiphenyls. This has also been demonstrated in fathead minnows exposed to Aroclors 1248 or 1260 for 66 days in water and transferred to PCB free-water for 34 days (DeFoe et al. 1978). Figure 6 shows a chromatogram of Aroclor 1248 residues in minnows after the 34 day washout period compared to an Aroclor 1248 standard. It can be seen that the composition of PCB residues in minnows is different

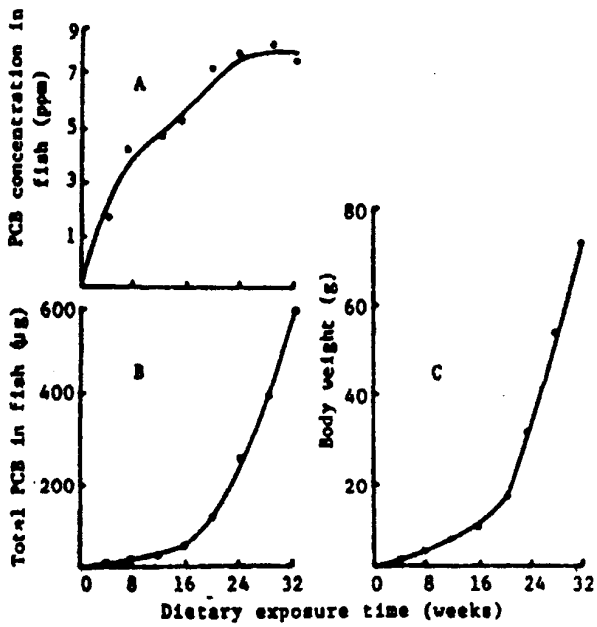

Figure 2. Bioaccumulation of Aroclor 1254 in rainbow trout following dietary exposure (15 ppm for 32 weeks).

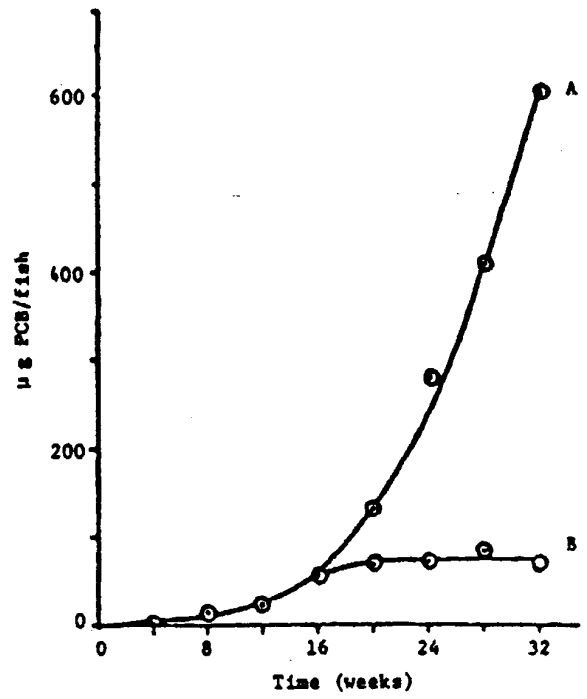

Figure 3. PCB concentration in rainbow trout following dietary exposure to 15 ppm Aroclor 1254. A. 32 week exposure; B. 16 week exposure followed by transfer to PCB-free diet.

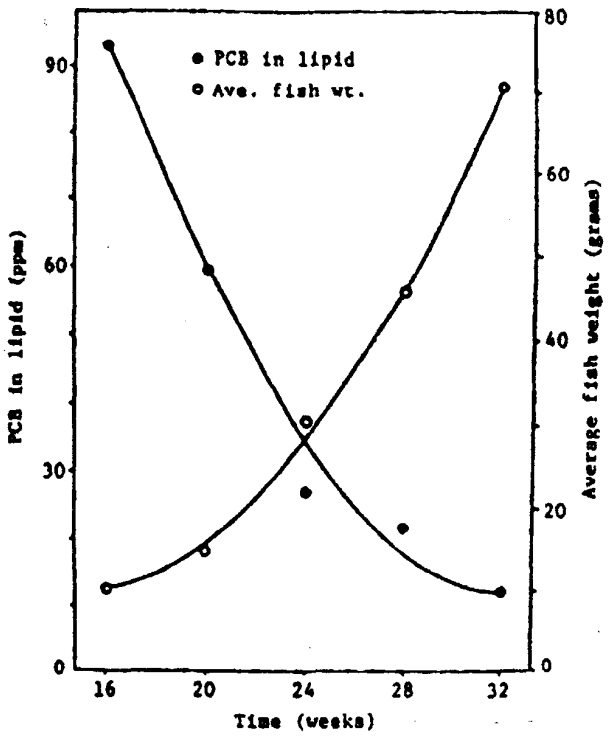

Figure 4. PCB concentration in lipid and weight of rainbow trout removed from diet containing 15 ppm Aroclor 1254 after 16 weeks.

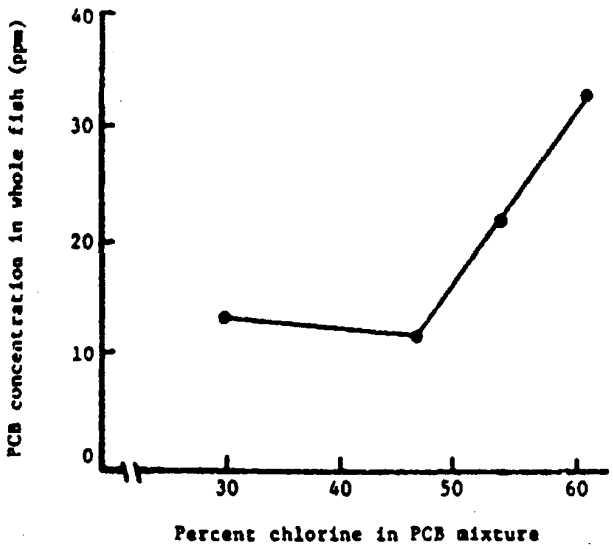

Figure 5. PCB concentration in channel catfish after dietary exposure to 24 µg/g Aroclors 1232, 1248, 1254 or 1260 for 193 days as related to the percent chlorine in each mixture.

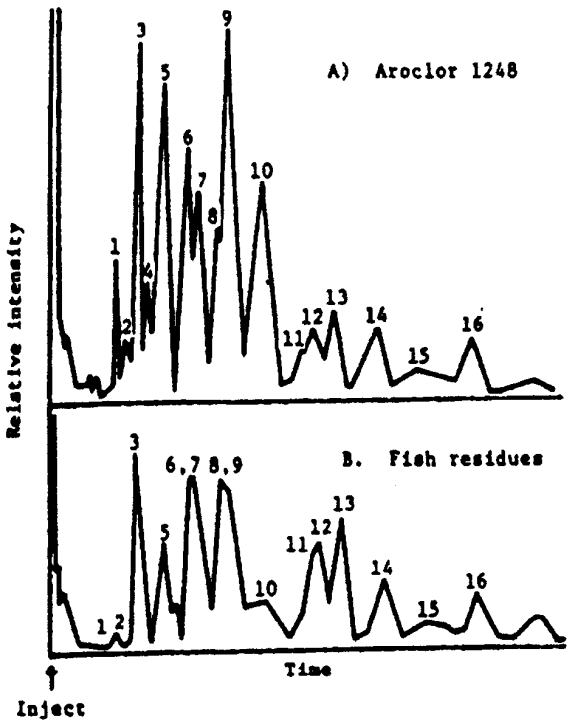

Figure 6. Gas chromatograms of (A) Aroclor 1248 standard; (B) residues in fathead minnows.

from the standard. A different result was obtained for Aroclor 1260 where PCB residues in minnows were essentially identical on a component basis to Aroclor 1260.

Along with biotransformation which may determine the extent to which PCBs can be accumulated by fish, the lipid content of the animal must also be considered. Female fathead minnows accumulated about twice as much Aroclor 1248 or 1260 from water as males and this was due to the higher lipid content in the female animals (DeFoe et al. 1978).

DISTRIBUTION

Mammals

Because of the many different chemical and biological properties of the individual congeners which make up commercial PCB mixtures, it has proven extremely difficult to assess the environmental fate and disposition of these chemicals. Thus, most studies to date have examined the tissue distribution and metabolism of individual PCB congeners. This has not only led to a better understanding of the pharmacokinetic behavior of polychlorinated biphenyls, but to the elucidation of the structure-activity relationships which determine their disposition and persistence. Administration of individual PCB congeners to laboratory animals has demonstrated that the lower chlorinated species are more rapidly metabolized than the higher chlorinated compounds and this contributes to the bioaccumulation of the latter in animal tissues. These types of studies have also shown that the tissue absorption and distribution of individual PCB components is both species and organ dependant.

The examination of the distribution and excretion of individual PCB congeners in rats has provided an insight into the fate of the more complex commercial PCB mixtures (Matthews and Anderson 1975, Lutz et al. 1977). The individual congeners studied included 4-chlorobiphenyl (1-CB), 4,4'-dichlorobiphenyl (2-CB), 2,4,5,2',5'-pentachlorobiphenyl (5-CB), and 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB). These particular congeners were chosen because their degrees of chlorination are similar to those of commercial PCB mixtures. It was determined that following intravenous injection of a single dose of each of the congeners, the PCBs were removed rapidly from the blood and initially stored in liver and muscle. More importantly, the rate of excretion or redistribution of the PCBs to tissues with high lipid content could be related to their degree of chlorination. Although the storage of metabolites did not occur to any appreciable extent, the percentage of total radioactivity accounted for by metabolites decreased as the percent chlorination of the particular congener increased. Ninety percent of the mono-, di- and pentachlorobiphenyls was excreted during the 42 day experimental period. The data further indicated that less than 20% of the administered dose of 6-CB would ever be excreted. These investigators thus suggest that the rates of elimination of PCB congeners are determined by their rates of metabolism.

The data in Figure 7 show the tissue levels of 6-CB after a single i.v. administration to the rat and those in Figure 8 show the cumulative daily excretion of 1-CB, 2-CB, 5-CB and 6-CB in feces. It is obvious that rates of metabolism of the individual PCB congeners play a role in their capacity for bioaccumulation and that this metabolism is dependent, at least in part, on

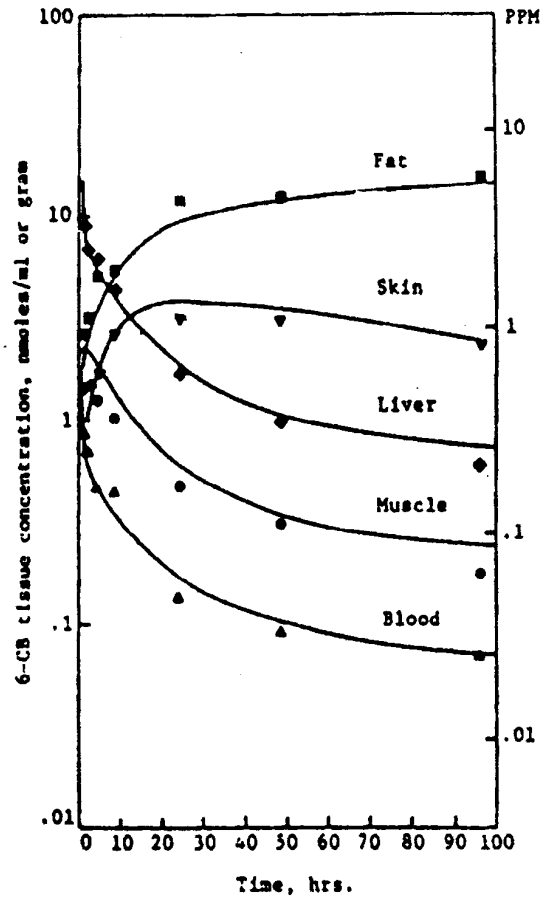

Figure 7. Tissue levels of 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) following a single i.v. injection of 0.6 mg/kg to male rats.

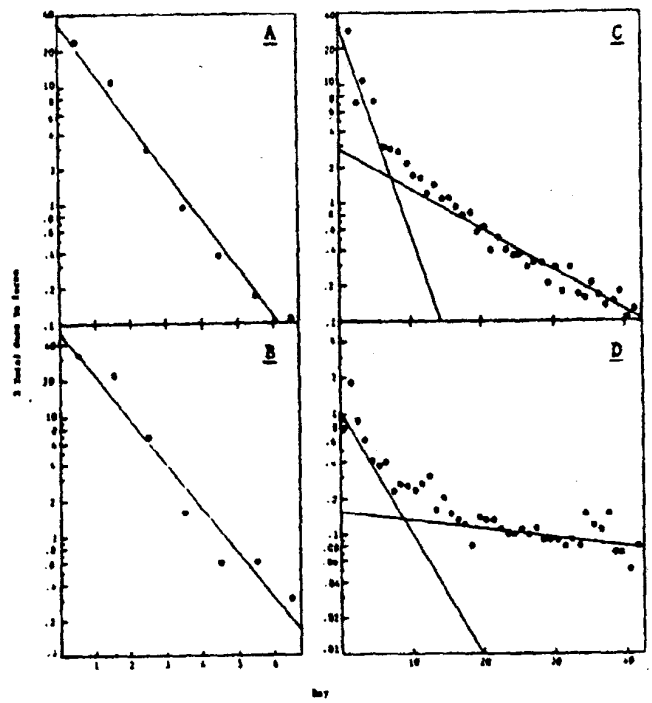

Figure 8. Percent of radioactivity of the total dose excreted in feces for 7 or 42 days following a single i.v. injection of 0.6 mg/kg of 1-CB (A), 2-CB (B), 5-CB (C) or 6-CB (D) to male rats.

the degree of chlorination. However, the position of the chlorine atoms on the biphenyl nucleus also determines the rate at which any congener is metabolized. Chlorine substitution patterns and their role in metabolism will be discussed in a later section.

The data gleaned from accumulation studies using polychlorinated biphenyl mixtures support those which have examined the behavior of individual congeners. Analyses of PCB residues from human tissues have demonstrated the retention of relatively few congeners when compared to the gas chromatographic profile of the commercial mixtures (Jensen and Sundstrom 1974, Yakushiji et al. 1979). Studies on the pharmacokinetics of commercial PCB mixtures in experimental animals have also demonstrated the retention of only certain congeners in adipose tissue (Hansen 1979).

Fish

Although a number of elegant studies have been performed investigating the tissue distribution of both commercial mixtures of PCBs and individual congeners in mammals, very few studies have been carried out on the disposition of PCBs in fish tissues. The data in Table 1 indicate the concentration of PCB residues and lipid content in tissues of coho salmon which were fed equal amounts of three chlorobiphenyls for 117 days. For most tissues examined, as lipid content increases, so does PCB concentration, although the data for brain, heart and spleen suggest that lipid content may not be the only determinant of PCB accumulation. Similar to what has been described in mammals, the more highly chlorinated congeners were found in higher concentration in the tissues examined.

The data of Guiney et al. (1977) suggest that disposition studies expressing only PCB concentration in various tissues may be misleading. The data in Figure 9 show the concentration of ^{14}C-labeled 2,5,2',5'-tetrachlorobiphenyl residues in tissues of rainbow trout for up to 56 days after water exposure. The highest concentrations of ^{14}C activity were in visceral fat, bile and eyes plus associated periorbital fat. However, when PCB residues in the same fish were expressed as a percentage of total ^{14}C content in the whole animal, it can be seen that carcass, skeletal muscle and skin contained the highest percentage of tetrachlorobiphenyl and visceral fat, eyes and periorbital fat and bile contained the lowest (Figure 10). These considerations of tissue mass should be taken into account in any disposition studies.

METABOLISM

Mammals

The role of metabolism in the capacity for bioaccumulation of PCBs has been alluded to previously, and the reader is referred to a recent, comprehensive review of their uptake, disposition, bioaccumulation and metabolism (Safe 1980). Briefly, those PCB congeners with higher chlorine content appear to be preferentially retained. Although routes of PCB metabolism can vary considerably depending on the species studied, a few generalizations can be put forth. The major metabolites of PCBs include phenolic products, dihydrodiols, and sulfur containing metabolites. Hydroxylation is favored at the para position in the least chlorinated phenyl ring. In the lower chlorinated

Table 1. Lipid Content and Concentration of PCB Residues in Coho Salmon Tissues[a]

Tissue	Lipid Content	2,4,6,2',4',6'-Hexachlorobiphenyl	2,4,5,2',5'-Pentachlorobiphenyl	3,4,3',4'-Tetrachlorobiphenyl
	(wt %)	(µg/g wet weight)		
Adipose tissue	73.0	18.80	19.30	10.60
Brain	7.1	0.38	0.31	0.15
Spinal column	6.6	1.40	1.40	0.92
Leteral line muscle	6.1	1.90	1.80	0.77
Intestines	4.3	0.82	0.79	0.30
Stomach and pyloric ceeca	3.3	0.93	0.76	0.43
Liver	2.9	0.50	0.35	0.25
White muscle	2.6	0.64	0.63	0.29
Heart	N.D[b]	1.50	1.20	0.98
Spleen	N.D.	2.10	2.00	0.85

[a]Gruger et al. 1975.
[b]Not detectable.

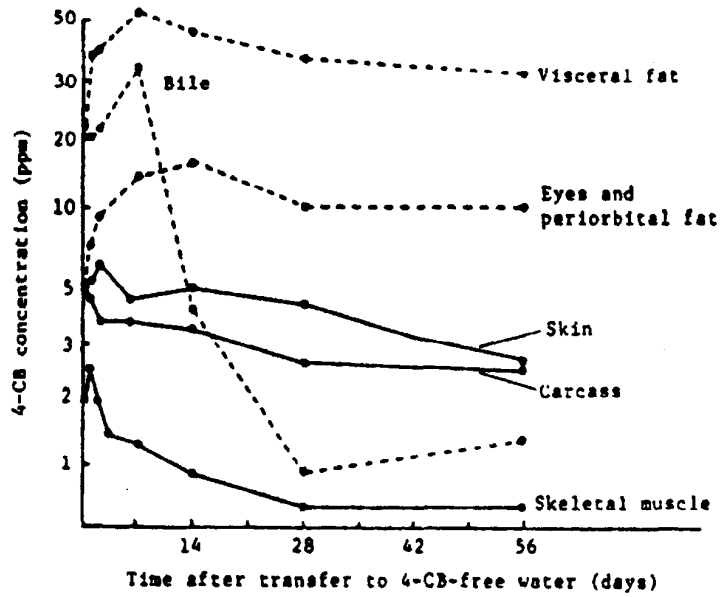

Figure 9. Tissue concentrations of ^{14}C in rainbow trout exposed to ^{14}C-4-CB in water and transferred to 4-CB-free water (day 0).

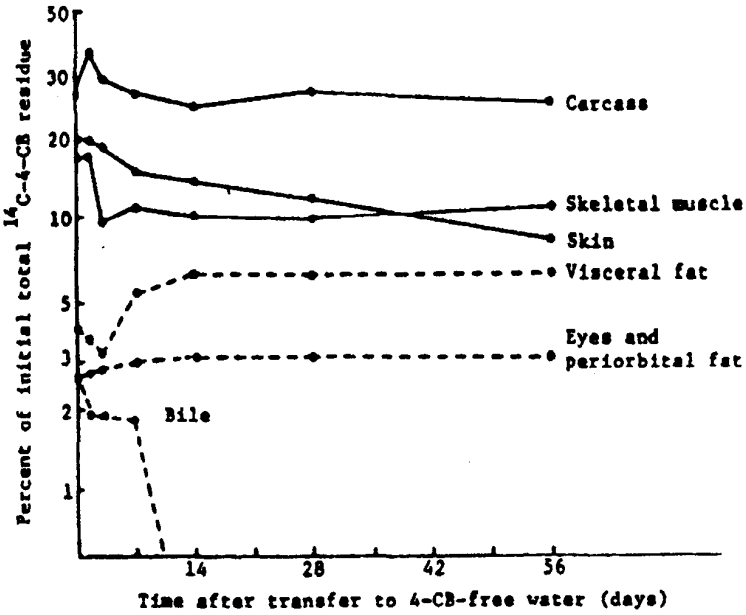

Figure 10. Percentage of total ^{14}C in tissues of rainbow trout exposed to ^{14}C-4-CB in water and transferred to 4-CB-free water (day 0).

biphenyl congeners, the para positions of both biphenyl rings and carbon atoms which are para to the chloro substituent are all readily hydroxylated. In general, as the degree of chlorination increases on both rings, the rate of metabolism decreases. Finally, oxidative metabolism of PCBs is further facilitated by the availability of two adjacent unsubstituted carbon atoms to allow arene oxide formation as an intermediate step in metabolism (Kato et al. 1980).

Although it has been definitively demonstrated that both the degree and position of chlorine atoms determines both the rates and routes of metabolism of PCB congeners, other factors may also be involved. Following examination of the pharmacokinetics of several tetrachlorobiphenyl isomers in the mouse, it was demonstrated that those isomers which possessed adjacent unsubstituted carbon atoms were more rapidly eliminated than those that did not, but rates of elimination of the former group of isomers were significantly different from each other (Sugiura et al. 1975, Sugiura et al. 1976). This led to the suggestion that PCB isomers with increasing ortho-chlorine substitution patterns are less planar and thus less soluble in fat tissue. Subsequent studies demonstrated that the accumulation of the more highly chlorinated PCBs is not directly proportional to lipophilicity (Hutzinger 1978, Tulp and Hutzinger 1978). Thus, although the number and position of chlorine atoms, and the octanol/water partition coefficients of PCB congeners may suggest their predisposition for metabolism, there does not appear to be one overriding factor which determines the rate of elimination of individual PCB congeners in mammals.

Fish

There is comparatively little information available concerning PCB metabolism in fish. Various investigations have demonstrated that fish are capable of metabolizing PCBs to compounds more polar than the parent (Sanborn et al. 1975, Metcalf et al. 1975, Bend et al. 1976, Hinz and Matsumura 1977) and the principal mechanism involved appears to be hydroxylation. Trout and skate, as well as the invertebrates, red crab and lobster, can metabolize biphenyl to both the 4-hydroxy and 2-hydroxy derivatives (Creaven et al. 1965, Gruger et al. 1975). Polar metabolites of ^{14}C-labeled 2,5,2',5'-tetrachlorobiphenyl were demonstrated in the bile of rainbow trout and one of these metabolites was identified as a glucuronide conjugate of 4-hydroxy-2,5,2',5'-tetrachlorobiphenyl (Melancon and Lech 1976). Dogfish metabolized 2,4,5,2',5'-pentachlorobiphenyl extremely slowly but polar metabolites were also found in the bile of these animals (Bend et al. 1976).

Although the effect of the position of chlorine atoms on the biphenyl nucleus on metabolism has not been studied in fish, an effect of the number of chlorines has been reported. Green sunfish were exposed to ^{14}C-labeled trichloro-, tetrachloro- and pentachlorobiphenyl congeners on days 0 and 9 and sacrificed on day 16, and the percent total radioactivity in fish as parent compound and polar metabolites was determined (Sanborn et al. 1975). The data in Table 2 indicate that, as in the mammalian species, the rate of metabolism appears to be inversely related to the degree of chlorination. Furthermore, these data suggest that the availability of more than two adjacent unsubstituted carbon atoms may be necessary for an appreciable rate of PCB metabolism. It should be noted that although some of the generalizations regarding the

Table 2. Effect of degree of chlorination on PCB metabolism in green sunfish[a]

PCB	% Parent	% Polar metabolites
2,5,2'-Trichloro	18	82
2,5,2',5'-Tetrachloro	99	1
2,4,5,2',5'-Pentachloro	99	1

[a]Sanborn et al. 1975.

metabolism of PCBs which have been determined from mammalian systems may be applicable to their metabolism in fish, fish metabolize PCBs much more slowly than their mammalian counterparts.

An attempt has been made to briefly discuss the factors which effect the bioaccumulation, persistence and metabolism of PCBs in a number of vertebrate species. Although a number of valid generalizations have been put forth regarding the pharmacokinetic behavior of PCBs, their fate and persistence in animals with altered physiological states has not been so comprehensively addressed. Of both environmental and toxicological concern is the effect of reproductive processes on the disposition, metabolism and elimination of these compounds. The remainder of this report will, therefore, deal with the effect of reproduction on the pharmacokinetics of specific PCB congeners in both fish and mammals.

Fish

2,5,2',5'-Tetrachlorobiphenyl (4-CB) has been shown to be one of the major PCB congeners in contaminated fish (Veith 1974). In adult rainbow trout exposed to this PCB congener in water, metabolism was slow as evidenced by less than 1% of the compound accumulated by the fish being isolated from bile in metabolized form (Melancon and Lech 1976). The effect of egg maturation on the distribution and elimination of 4-CB residues in female rainbow trout was studied by exposing two year old fish to ^{14}C-labeled 4-CB in water for 36 hours in December with subsequent transfer to flowing, non-recirculating pristine water (Guiney et al. 1979). At designated times thereafter, fish were sacrificed and selected tissues analyzed for radioactivity. In these animals, vitellogenesis was initiated in June and spawning commenced in October. Figure 11 shows the elimination of 4-CB from female trout on a whole body basis and weight of maturing eggs at each sampling time. From January through August, the elimination of 4-CB was slow with an apparent t 1/2 of elimination of 1.76 years. Wet weight of the eggs was low from January through May, then increased gradually from the end of June through August. Beginning in September, there was a rapid increase in egg weight which reached a peak in October. The mature eggs were then spent from the fish causing the weight of the eggs which remained in the peritoneal cavity to decrease dramatically by December. The vertical dotted line indicates the last sampling time before the rate of 4-CB elimination from the whole fish appeared to increase. Using this as a reference point for the change in 4-CB elimination rate, it can be seen that the increased elimination occurred at the end of egg maturation and throughout the spawning season. The t 1/2 of elimination was reduced to 0.5 years.

The relationship between egg maturation in female trout and 4-CB content of the eggs is given in Figure 12. The major finding was that as egg weight increased to a peak, the percent ^{14}C residue in the eggs also increased. When the female ovulated and the eggs were spent from the fish in the act of spawning, the 4-CB associated with the eggs was eliminated from the body. This resulted in a reduced total percent ^{14}C residue associated with the fewer eggs that remained in the peritoneal cavity by December when the spawning season was over. Taken together, these findings suggest that elimination of 4-CB in the spent eggs was, in part, responsible for the more rapid elimination of 4-CB from female rainbow trout from September to December.

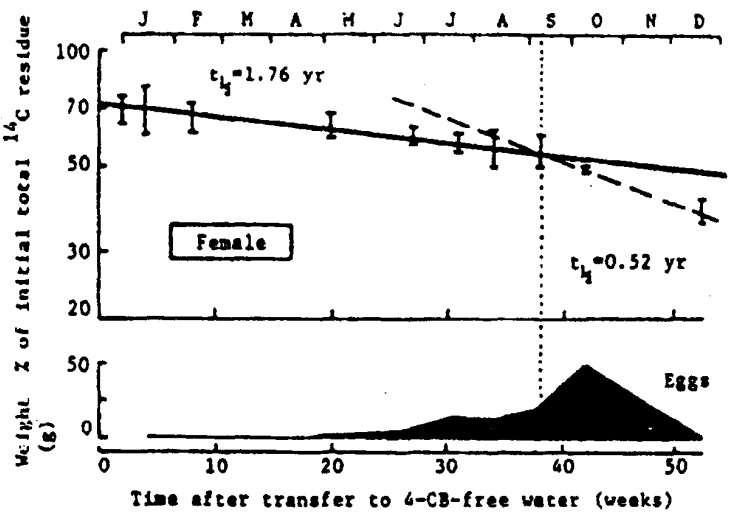

Figure 11. Effect of spawning on elimination of ^{14}C-4-CB residues in female rainbow trout and wet weight of eggs following transfer of animals to 4-CB-free water (day 0). The vertical line represents the last group of fish sampled before elimination rate increased.

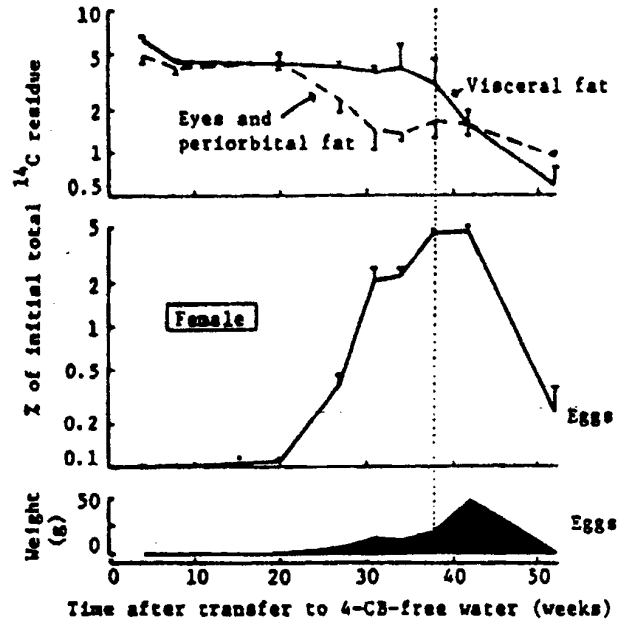

Figure 12. Redistribution of ^{14}C activity from fat depots to eggs of female rainbow trout following transfer of animals to 4-CB-free water (day 0), expressed as a percentage of whole body ^{14}C-4-CB residues.

Although the 4-CB that accumulated in the eggs may have originated from a redistribution of 4-CB from several compartments of the body, a temporal relationship was found between the accumulation of 4-CB in maturing eggs and the depletion of 4-CB from body fat. More explicitly, the increase in egg 4-CB content was associated initially with a reduction in 4-CB content of eyes and periorbital fat. This was followed later in time by a decrease in 4-CB content of visceral fat. It is probable therefore, that some of the 4-CB accumulated in maturing eggs was derived from these body fat depots.

Generally, wet weight of eggs, eyes plus periorbital fat and visceral fat increased after transfer of female trout to 4-CB-free water but the increase in wet weight of the maturing eggs far exceeded that of visceral fat and eyes plus periorbital fat. 4-CB concentration in the eggs remained essentially constant throughout the study, while that of visceral fat and eyes plus periorbital fat decreased. The reduction of 4-CB concentration in these latter two body fat depots cannot be explained solely on the basis of an increase in fat wet weight, demonstrating that other factors were also involved.

Johnson and Morris (1974) found a direct linear relationship between the PCB and lipid content of eggs stripped from freshwater fish in nature. This suggests that lipid content of the eggs is a determinant of the magnitude to which PCB accumulates in them. What is less clearly understood is the mechanism of the depletion of 4-CB from visceral fat and periorbital fat and eyes in the present study. The observation that the wet weight of visceral fat, eyes, and periorbital fat remained virtually constant during the time that 4-CB content of these tissues was decreasing indicates that the redistribution of 4-CB is not due to the redistribution of body fat.

In some migratory salmonids which do not feed during the spawning run, the percentage of oil in the whole fish drops to less than 10% of the pre-spawning value (Love 1970). Under these conditions, the percent PCB body burden transferred to the eggs may be even greater. These data suggest that spawning may provide an alternate route of elimination of persistent PCB congeners in fish species.

Mammals

Whether similar alterations occur in the pharmacokinetics of a persistent PCB congener during reproduction in mammals was also examined. A number of studies have been carried out on the transplacental passage of polychlorinated biphenyls and their transfer to nursing offspring. In view of the fact that numerous recent monitoring studies have demonstrated the presence of PCBs in human breast milk in various parts of the world (Yakushiji et al. 1978, Brevik and Bjerk 1978, Kuwabara et al. 1979, Mes and Davies 1979), it is surprising to note the paucity of toxicological information regarding their half-lives and disposition in pregnant and lactating animals and their rate of accumulation and disposition in offspring.

Previous investigations in rodents with PCB mixtures have demonstrated minimal transplacental passage of these chemicals when compared to levels observed in the milk of lactating mothers (Curley et al. 1973, Takagi et al. 1976, Masuda et al. 1978). However, use of mixtures of PCBs does not allow for an accurate appraisal of the behavior of those particular congeners known

to be the most persistent. As mentioned previously, the data of Matthews and Anderson (1975) suggest that less than 20% of the total administered dose of 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) would ever be excreted from male rats. 6-CB was accumulated primarily by adipose tissue, muscle and skin in these animals. Also, Jensen and Sundstrom (1974) have shown 6-CB to be the most prevalent PCB in human tissue samples. It was thus of interest to evaluate whether this persistent PCB congener was transferred transplacentally or to nursing offspring through milk.

Although the minimal transplacental transfer of 6-CB has been reported in mice (Orberg 1977), this study did not consider the distribution of the PCB in mothers, rates of elimination in milk or disposition and rates of accumulation in offspring. As is also the case in the aforementioned investigations with PCB mixtures, animals in this experiment were dosed chronically during pregnancy and/or lactation and thus transfer of 6-CB from storage depots in the mother could not be determined. These studies do not reflect the situation in which exposure to PCBs results in the selective sequestration of particular congeners into storage depots prior to pregnancy through the metabolism and/or elimination of less persistent congeners. We have thus studied the disposition of 6-CB in pregnant and lactating mice and its accumulation in the fetus and nursing offspring after administration of ^{14}C-labeled 6-CB to female mice 14 days prior to mating the animals (Vodicnik and Lech 1980). The data in Figure 13 show that no significant differences were noted in the disposition of ^{14}C activity in the tissues from pregnant animals when compared to virgin controls. On the day of birth however, liver, adipose tissue and kidney 6-CB concentrations were significantly higher in mothers than in virgins sacrificed at the same time. The half-lives of elimination of 6-CB in adipose tissue in 1-to 12-day pregnant mice and their virgin controls were 7.5 and 11.6 days, respectively, and in the liver, 17.5 and 15.8 days, respectively.

During lactation, 6-CB concentration decreased sharply in all tissues sampled from mothers (Figures 13, 14). Tissue concentrations in virgin controls sacrificed at the same time remained elevated at levels similar to those of virgins sacrificed concurrently with day 12 pregnant animals. The half-life of elimination of 6-CB from the adipose tissue of virgins sacrificed following the equivalent of the 12th day of pregnancy was greater than 100 days. The half-life in the liver of virgins could not be determined due to the lack of detectable elimination of the chemical after this time. Half-lives of elimination from liver and adipose tissue fell to 4.7 and 2.1 days, respectively, in lactating mothers.

Little 6-CB was transferred across the placenta during the course of pregnancy. The concentrations of 6-CB in fetuses from 12 and 18 day pregnant animals were 0.71 and 2.45 µg/g tissue, respectively. At birth, total carcass concentration of 6-CB in pooled newborns was less than 3 µg/g tissue which represents less than 3% of the body burden in the mothers at birth.

Throughout the course of lactation, suckling offspring accumulated 6-CB through milk. Figure 14 shows the concentration of 6-CB in the mammary glands of late pregnant and lactating mice. These data closely parallel those of stomach contents concentrations of 6-CB in nursing offspring. The PCB accumu-

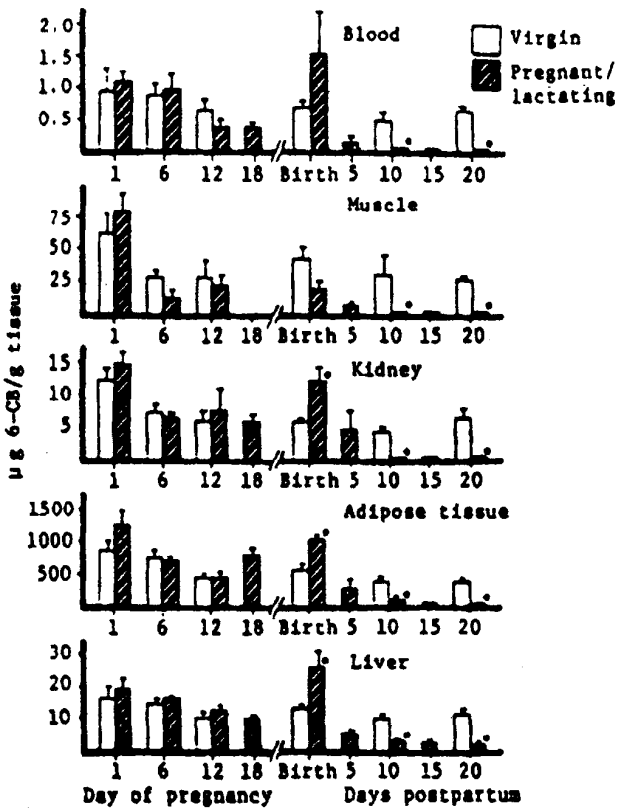

Figure 13. Concentration of ^{14}C-6-CB in selected tissues from virgin and pregnant/lactating mice. * represents significant difference from virgin control ($p < 0.05$).

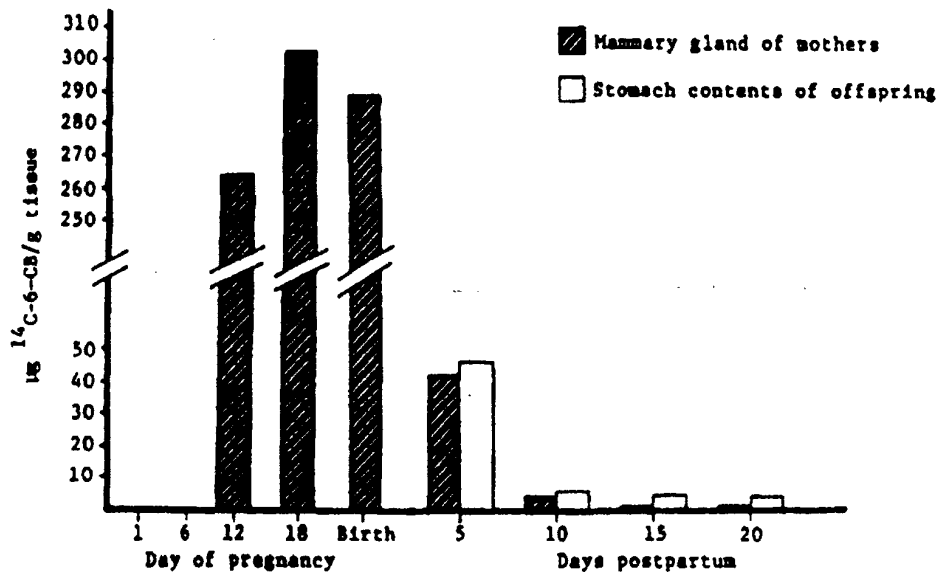

Figure 14. Concentration of ^{14}C-6-CB in the mammary gland of pregnant/lactating mice and stomach contents of nursing offspring.

leted primarily in the liver, muscle and skin of suckling young. Adipose tissue samples could not be obtained from offspring in great enough quantity to allow for reliable ^{14}C determination. Figure 15 shows that levels of 6-CB were highest in the liver of offspring at day 5 postpartum and hepatic content dropped sharply by 10 days postpartum. Muscle and skin concentrations of 6-CB reached a maximum at day 10 postpartum and decreased thereafter, corresponding to the increase in litter weight and decrease in 6-CB concentration in milk. From the design of this experiment, it was not possible to distinguish whether the decreased concentration of 6-CB was the result of elimination or simply a dilution effect due to rapid growth of the offspring at this time.

In order to determine the percentage body burden of 6-CB eliminated during lactation, virgin female mice were injected with ^{14}C-6-CB 14 days prior to mating and two animals were sacrificed on day 19 of pregnancy and two mothers and their litters were sacrificed 5, 10, 15 and 20 days postpartum. ^{14}C activity was assessed in carcasses of mothers and their pooled litters. The data in Table 3 show total carcass 6-CB content in late pregnant and lactating mothers and their offspring. Mothers eliminated 57% of the total dose of 6-CB remaining during late pregnancy by day 5 postpartum and essentially their entire body burden was eliminated by day 20 (> 98%). Nursing offspring accumulated 6-CB through milk and all of the ^{14}C eliminated by the mothers could be accounted for in the suckling offspring.

These data support those of other investigators who have suggested the placenta to be an effective barrier to the passage of PCBs in experimental animals whereas the mammary gland represents an important route of elimination (Curley et al. 1973, Orberg 1977, Masuda et al. 1978). These results also support the human data of Masuda and coworkers (1978) who demonstrated that although PCB levels in the blood of stillborn fetuses were much lower than those in their mothers, nursing infants from the same population had higher levels of PCBs in blood than those in maternal blood.

The higher concentrations of 6-CB in adipose tissue, liver and kidney in mothers on the day of birth in this study may be the result of lipid mobilization to the proliferating mammary glands. That is, mammary gland development and/or milk production may be occurring, in part, at the expense of stored adipose tissue lipid during late pregnancy resulting in a further concentration of 6-CB in fat. The increase in liver and kidney 6-CB content at this time suggests some translocation of the chemical to the developing mammary gland so that these tissues may have been exposed to more circulating PCB. Although the concentration of 6-CB was not significantly different in the blood of virgin and pregnant animals, it is possible that there was an increased turnover of 6-CB in the blood. In support of this hypothesis is the fact that PCBs have been shown to become associated with plasma lipoproteins in vitro (Matthews et al. 1977, Vomachka et al. 1983) and that mammary gland lipoprotein lipase activity increases and adipose tissue lipoprotein lipase activity decreases during lactation to facilitate triacylglycerol incorporation into milk from chylomicrons and very low density lipoproteins (Zinder et al. 1974).

Whether rates of elimination and the percentage body burden of 6-CB eliminated through breast milk in humans are similar to those parameters observed in mice cannot be ascertained. Elimination through the lactating

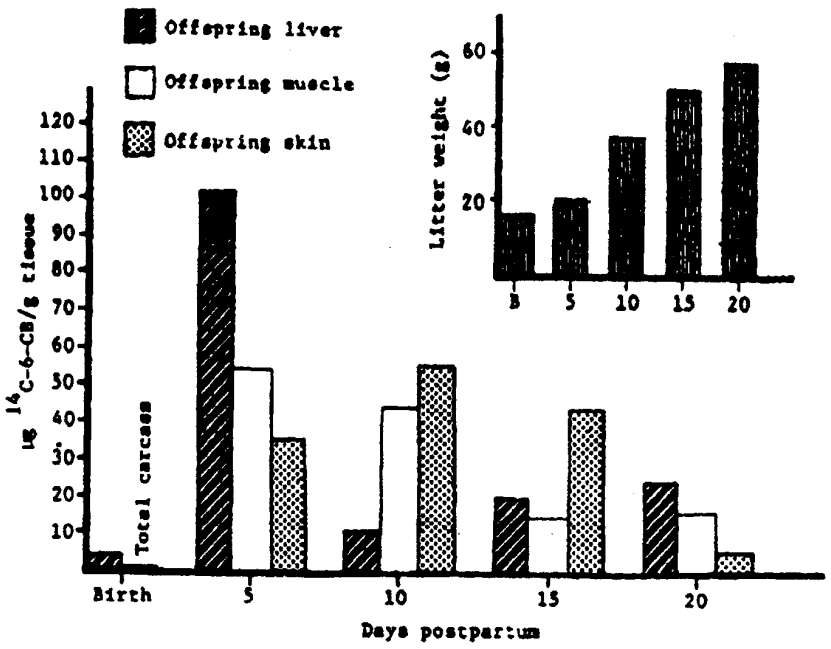

Figure 15. Concentration of ^{14}C-6-CB in tissues from offspring of ^{14}C-6-CB pretreated-mothers. Inset Mean litter weights on days of sacrifice.

Table 3. Transfer of [^{14}C]6-CB from mothers to nursing offspring

Day of sacrifice	Mothers[a]			Offspring[c]		
	mg 6-CB/ total carcass	µg 6-CB/ g carcass	Percentage total dose eliminated[b]	mg 6-CB/ litter	µg 6-CB/ g litter	Percentage of mother's dose accumulated
Day 19 pregnancy	0.862	15.12	0	----	----	----
Birth	----	----	2.7[d]	----	----	3.00[d]
Day 5 postpartum	0.372	8.59	56.8	0.545	15.31	63.2
Day 10 postpartum	0.167	3.48	80.6	0.810	13.97	94.0
Day 15 postpartum	0.038	0.87	95.6	0.743	11.18	86.2
Day 20 postpartum	0.016	0.37	98.1	0.900	12.50	104.4

[a]Virgin female mice pretreated with [^{14}C]6-CB (approximately 1.25 mg/animal) two weeks prior to mating.
[b]Each value represents the mean of two carcasses.
[c]Represents percentage eliminated from dose remaining in mothers at Day 19 of pregnancy.
[d]Each value represents the mean from the carcasses of two pooled litters.
[d]Approximate, from Figure 15.

mammary gland may not only depend on the number of nursing offspring and diet of the mother, but also on the dependence of a particular species on dietary versus stored lipid for the production of milk triacylglycerol. We have observed that parametrial fat stores were significantly depleted in 20 day postpartum mice nursing average sized litters. Milk fat content also differs among species. It has been demonstrated that human milk contains approximately 4% lipids, whereas mouse milk contains up to 13% milk fat (Jenness 1974).

We have recently obtained similar results following the administration of 3,4,5,3',4',5'-hexachlorobiphenyl to virgin female mice (Vodicnik and Lech 1982). This latter compound was sequestered into storage depots and minimal transplacental passage was observed following mating of these animals. However, mothers eliminated nearly 100% of their body burden to nursing offspring, and the bulk of eliminated ^{14}C activity could be accounted for upon analysis of pooled litter carcasses. These data suggest that the pharmacokinetic behavior observed was not dependent on the planarity of the PCB isomer.

These investigations with tetrachlorobiphenyl in the rainbow trout and two hexachlorobiphenyl isomers in the mouse indicate that once persistent PCBs are sequestered into storage depots, they can be redistributed to other lipid-rich tissues (milk, yolk) and thus be eliminated from the animal via alternate routes. In both fish and mammals, the physiological processes associated with reproduction offer alternative routes of elimination which can significantly affect the pharmacokinetic behavior of PCBs.

The mechanism(s) by which the transfer of PCBs from storage sites in the mother to developing oocytes in fish or milk in mammals occurs remains unknown, but it is possible that the endocrine changes associated with vitellogenesis or the onset and maintenance of lactation may be involved. Estrogen and prolactin in mammals and estrogen in fishes are responsible for the biochemical and physiological alterations observed during lactation in mammals and during prespawning and spawning in fish, and have also been shown to alter plasma lipoprotein profiles and/or turnover. Since PCBs have been shown to associate with plasma lipoproteins (Mattheve et al. 1977, Vomachka et al. 1983), and since plasma lipoprotein profiles are altered during vitellogenesis in fish and late pregnancy in mammals, this phenomenon may be, in part, responsible for the observed redistribution of PCBs described in the present studies. In conclusion, the physiological adaptations associated with reproduction in both fish and mammalian species may profoundly alter the distribution and elimination of selected PCB congeners. We are presently attempting to assess the possible role of plasma lipoproteins acting as carriers of PCBs from sites of storage to the developing oocyte in fish and mammary gland in mammals.

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DISCUSSION SUMMARY

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DR. MELANCON: Obviously the PCB problem has related very much to the aquatic environment because this is a route by which the PCBs have reached some humans and the way the problem has come to attention. The presentation referring in large extent to the Great Lakes fish earlier shows this strong tie-in to the aquatic environment.

The explanation for the disappearance of the lower chlorinated PCB congeners was very interesting and relates to the pattern we are aware of that the lower chlorinated isomers tend to disappear. In fact, even beyond whatever degradation occurs in river water, we are aware that many species of animals are capable of metabolizing the lesser chlorinated isomers, while fish cannot metabolize the higher chlorinated isomers.

Earlier we were talking quite a bit about analytical work and the significance of PCB determinations. In regard to the data from the Michigan Conservation Department, it would be nice if we could see the statistical evaluation. Maybe this can be commented on later.

Another question which came to my mind is if the total PCBs are dropping somewhat, is this actually a drop in PCBs in general or just the disappearance of the lesser chlorinated isomers; is the pattern of isomers in the fish changing from what it was before?

We have discussed mainly the Great Lakes this morning. What is happening to PCBs in other aquatic systems across the United States? I know the Department of the Interior has a study on the Mississippi River (the upper Mississippi River is a problem area for the PCBs) and has a nationwide testing program which I believe covers a period from 1973 to 1979. Maybe someone could comment on this later.

The quantity of PCBs reaching the environment per year may be less now because of the various controls and the decreased production of PCBs as PCBs per se rather than as impurities as presently. Currently, the relatively large quantities of PCBs present in sediments may, in effect, be a sink whereby they are relatively inactive. On the other hand, it may be a reservoir from which in the future they can keep supplying the environment with PCBs even though the amount of new material coming in is less. Are these sediments sinks or are they going to be problems, say, in rivers because they can be stirred up at various times of the year, spring floods, or when harbors are dredged and so on? This is something we have to consider for the future.

A brief comment on the second presentation, the reproduction-related PCB redistribution which can occur in vertebrates as different as fish and small mammals. It is interesting that under normal conditions fish cannot do much to

eliminate higher chlorinated PCB isomers rapidly (T 1/2 = 1.5 or 2 years, probably higher for the more highly chlorinated isomers), yet during sexual maturation, egg formation and so on, PCB isomers can be transferred to the eggs and more rapidly eliminated. In the small mammals, the fetus does not receive the PCB isomers. There were a couple of possible reasons given for that, yet during nursing there is a transfer from the mother to the milk and to the offspring. Presumably if PCBs were being given at the time that the eggs were being formed or nursing going on, these PCBs too, might be transferred.

If anyone would like to start off the questioning now or make comments, you are welcome to.

DR. KIMBROUGH: I actually have several questions. First of all I would like to discuss the results with the excretion of PCBs through milk and the relevance of that to humans. Of course, you cannot do these types of studies in humans. So, what we have primarily done is looked at PCBs in milk, in adipose tissue and in serum. Often these samples were not collected from the same type of people. We all carry very low body burdens of PCBs and most women will excrete PCBs in milk. On the other hand, there has been the recommendation that women ought to continue nursing because of the benefits of nursing. One reason for doing that is that the concentration in milk has always been very low and also, in looking at successive pregnancies and the little bit of data that we have, there does not seem to be this tremendous mobilization in people. They would never rid their body burdens in just nursing, and as was already pointed out, one reason is the difference in the lipid concentration in milk from different species. In the mouse it is 13 percent, I think it is 15 percent in the rat, and in humans it ranges somewhere from one to four percent. It also depends on where in the nursing period you take the milk sample, the amount of milk that is being produced, and the growth rate of the offspring. Mice and rats have a tremendous growth rate compared to the growth rate of an infant over a whole year. The period of nursing in humans is also much different. So, there are a lot of differences, and if you look at the USDA data, for instance, where there were successive pregnancies, women produced the so-called "cola" colored babies. There were several successive pregnancies where the second infant was, also, a cola colored baby. So, that is, in a sort of a roundabout way, again a suggestion that the women really don't mobilize a lot of this material, and I was wondering if anyone could comment on that?

DR. SAFE: Could I make a comment on that? There is a paper out by a group in Japan which looked at an occupationally exposed mother. I think it was published in the Bulletin of Environmental Contamination, and in that particular study the woman excreted a considerable part of her body burden of PCBs in milk (Yakushiji et al. 1978).

DR. BELL: (General Electric). In that study, over 16 months of lactation, the mother eliminated 75 percent of her body burden of PCB, and that was confirmed in the milk which was collected and analyzed.

DR. VODICNIK: Was there any estimate in that study of the amount of fat in the mother? Was there a significant weight decrease? Was lipid being mobilized?

DR. BELL: In the paper itself that is not commented on, but I understand from other private conversations that the lipid in the woman did not change substantially over the period of lactation. So, I don't think we are looking at a transfer simply because she is eliminating her lipid. I think we are seeing a washing out of PCB into her mammary gland and then into the milk produced.

DR. JASON: Casey Jason from EPA. There is one other report. -- Wickheiser recently reported in the American Journal of Public Health of the Michigan group of over 2000 women that were being sampled for PBB levels which was done for free. Those willing to pay had PCB levels determined also. This was a little over 1000 women. As it turned out, it was very interesting. In looking at the breast milk, PCB levels in half of the women approached the tolerance limit of the FDA which was 1.5 parts per million. He also calculated the average amount of breast milk taken in by an infant in an 8-month period, in this group of women. Given a 50th percentile growth rate in the child, and assuming there is no affect from anything the child is taking in exogenously from the environment, this child should approach a one part per million level by eight months of age.

DR. WOLFF: One thing about the Japanese study you are talking about is that this woman nursed for 16 months, an unusually long period, and it was also a very large volume of breast milk. In any case, certainly the body burden could have been reduced.

I want to comment on the generalization that the lower chlorinated biphenyls are not bioaccumulated. I think this is sort of a statistical event. First of all, there are not as many isomers among the trichlorobiphenyls that are appropriately substituted, and I think that it is much more important to concentrate on that. Also the amount of so-called "accumulation" depends on how recent the exposure was. In the people that we have looked at that were occupationally exposed to the lower chlorinated biphenyls, by far the greatest PCB congener in those people was the trichlorobiphenyl 2,4,4'- which was also the one that had the highest concentration in the chlorobiphenyl mixture to which they were exposed.

DR. STALLING: I wanted to briefly summarize some information that will be in a publication sometime in the not too distant future in Pesticide Monitoring Journal, from the monitoring of fish residues of PCBs. There are 100 sampling sites in the US, and this country-wide data to some extent parallels but to some extent does not parallel the Lake Michigan situation.

The occurrence of PCBs apparently increased between 1974 and 1978/79. They were found at 93 percent of the stations sampled in 1974, 92 percent in 1976/77 and 98 percent in 1978/79. Maximum concentrations did not change appreciably. Concentrations approaching 100 parts per million wet weight, and 500 micrograms per gram lipid weight were measured as recently as 1978. Of the four PCB mixtures measured, that is Aroclors 1242, 1248, 1254 and 1260, 1254 was most widespread. It was found at 84 percent of the stations in 1976/77, 98 percent in 1978/79; residues resembling 1260 were present at 84 percent of the stations in 1976, 88 percent in 1978, and Aroclor 1248 was present at 39 percent of the stations in 1976 and 42 percent in 1978/79. No results resembling Aroclor 1242 were found in 1976/77, down from about 14 percent reported in 1973 and about five percent in 1974.

DR. WEBBER: Ian Webber, RTE Corporation. Have you looked at the metabolic products of PCBs either in the environment or through animals? The reason that I asked that question was because I did some work with the Canadian Electrical Association on trying to eliminate PCBs from insulating oils using strontium 90 high-energy beta particles. There is a correlation between using high-energy electrons from beta particles, from a Van de Graaff machine which MIT did, using cobalt 60 rays which the Japanese triad or using a chemical process like elemental sodium, to generate electrons.

In the environment you would tend to get e^- aqueous attaching to the water and splitting that as OH radicals and H radicals.

My system involved PCBs in oil with isopropanol and potassium hydroxide. I found that the isopropanol acted as a catalyst and the potassium hydroxide was involved in the overall chain by producing isopropoxide radicals. The PCB became a radical anion which eliminated chlorine as chloride and gradually degraded, attaching hydrogen from the isopropanol and eventually ending up as biphenyl. In the process though you get OH radicals. There is a competing reaction between the OH radicals and H dot. Now, the H dot because of its mobility tends to get to other radicals much faster, and therefore you end up with biphenyl.

In some of the cases, a substantial portion of the cases, you do find that hydroxylation has taken place. So, the hydroxylation then produces water solubility of the otherwise insoluble PCB. If you then look at the oil, you will find that you have interacted with all the PCB, and you no longer have PCB contained in that oil. However, the water layer now contains a far worse problem than you started with because of the hydroxylated product. It can go from that by elimination of HCL if it is suitably oriented on the PCB molecule to produce dibenzofurans, and then we come back to that problem. So I dropped that work because of the generation of dibenzofurans and a worse problem than I started with.

DR. MOOLENAAR: Your question related, I think, to the products of degradation of PCBs in the environment, and I think you saw from the talk that we have studied in some detail the products that might be produced, intermediate products in the biodegradation by microorganisms of cholorobiphenyls.

Not only have we done that, but the University of Michigan workers have as well, and a consistent picture emerges that really the only isolatable intermediates are the chlorobenzoic acids which then degrade further.

If one thinks of the degradation of these materials in the atmosphere which is probably initiated by OH attack, you would expect the same sort of a degradation pattern for these materials as you see for hydrocarbons in general, and indeed in the Applaby experiment from several years ago they did note some aldehydes and so on as intermediate degradation products in that study, but most of those, of course, are also subject to OH attack. You would expect the progression of degradation similar to what you see for hydrocarbons that enter the atmosphere.

In terms of water degradation involving free radicals, this has been a subject of some study over the last few years, but as far as I know in most

cases this route of degradation is not very important from an environmental point of view for anything that has a half life of less than one year or so. Although the individual reactions may be fast, the concentrations of radicals in the water phase is very low, and so they do not seem to be very important reactions from an environmental point of view. I would be very surprised if you would see much of that going on with the chlorobiphenyls in a water system.

You mentioned the hydroxylation of the chlorobiphenyl. If that were to occur, forming a compound which is now hydroxylated, we have increased the water solubility quite a bit, and those kinds of materials are known to be fairly biodegradable. The phenols and the phenyl substituted phenols are fairly rapidly biodegraded, much more rapidly even than the chlorobenzoic acids themselves. So, if hydroxylation were to occur I would expect them to degrade rapidly via the microbial process.

DR. VODICNIK: I would like to make a comment on your data with the chub in Lake Michigan. I believe it showed the concentration of PCBs decreasing over a six or seven-year period. I think depending on the way the animal was exposed one has to consider in any expression of bioaccumulation the growth of the animal. I don't know what the growth rate of the chub is, but if the animal, for example, was exposed to a spill and accumulated a good deal of PCBs and then grew there would not be an elimination of the PCB from the animal. There would just be a dilution. So, while the concentration in any particular tissue might have decreased, the amount in the animal may not have decreased.

However, looking at it from the other sense where you see the decrease in concentration, it could relate to the spawning data suggesting that again the PCBs are being diluted out. A rainbow trout is capable of spawning up to 10,000 eggs. If there is a significant amount in the eggs and that PCB is retained by the offspring there could be that dilution effect again. So, you may not be getting a decrease of PCB in the biomass, but you are getting a decrease in the concentration in any particular animal.

DR. MOOLENAAR: Thank you for that comment. I think your point is well taken and that is the very reason why I think the State of Michigan biologists and those that work in the other laboratory that is involved have selected the chub as their test species. These are small fish, are relatively short-lived, and the sampling times, locations and the size of the fish taken and so on were all carefully selected with these kinds of parameters in mind because they wanted to get a true representation of what they thought the fish population was doing as a function of time in the lake. All of these factors were carefully analyzed, and the biologists there feel that they are truly getting a representative sample of what would be expected to be occurring as a function of time in Lake Michigan.

DR. KIMBROUGH: I have a comment and a question. As far as nursing and the nursing period in humans is concerned, from telephone calls that I get it is not unusual in the United States now that women will nurse for 16 months to two years. Dr. Moolenaar, you briefly mentioned in passing that there was a difference in the biodegradation of different isomers, and I was wondering if you could comment on that a little bit more. Is that the same as we find with metabolism, and which are the isomers that are the most persistent?

DR. MOOLENAAR: In regard to your question on the biodegradability of the different isomers, I don't recall all of that information in the literature precisely, but it is my general impression that the pattern you see in mammalian systems of degradability when you have adjacent carbons without chlorines is what you see in microbial systems, and so I believe the pattern there is fairly consistent.

DR. SAFE: I would like to make one comment about bioaccumulation. We published a study a couple of years ago in which we used five hexachlorobiphenyls, none of which had two adjacent unsubstituted carbons. They were hexachlorobiphenyls, so they were relatively nonmetabolizable, and they all had chlorines on the #4 positions of both rings. These are chemicals you would look at and say, "They are not going to be metabolized very quickly." The difference in the five isomers was in their degree of ortho-substitution, and in a sense as we decrease ortho-substitution, we tend to increase toxicity. In that study, which was a very preliminary 30-day study, we used five different species of animals. The animals that preferentially bioconcentrated the planar or lower ortho-substituted isomers were the animals more susceptible to PCB toxicity. For example, in the quail and in the guinea pig there was a very high concentration of the highly toxic 3,4,5,3'4'-5'-hexachlorobiphenyl and in the quail the funny thing about it is that it was the only compound of the five that was left.

If you look at the rat, the fish and the rabbit, the five isomers were present in about equal concentrations. There seems to be another factor not associated with metabolism but associated with shape and of course, shape of the molecule is associated with toxicity. So, that is another factor that one has to consider, and it is species dependent.

DR. BROOKS: Ronald Brooke, G.E. Regarding the comment of the parallel correlation between microbial degradation and mammalian, I think it is important to remember that in microbial systems, the literature is not very extensive regarding specific isomers challenged with either mixed or pure cultures. Secondly, in those cases where there have been, rates have not been a primary concern. What has been a concern is whether the molecule degraded at all and whether there were metabolites produced. Finally, it is important to remember that it has not yet been established that all isomers enter into or are available for microbial degradation and so with the microbial system you have to worry about both solubility and transport and the presence of a suitable activity in the organisms. Of the 33 isomers which have been examined there is noticeable difference among various strains of bacteria, so that in order to make some specific statement additional work has to be done.

DR. MOOLENAAR: That is an excellent comment, and I think it is really good that in our first presentation this morning we heard of the work going on in the analytical area to better determine these individual isomers. That has really been a holdup in a lot of the studies that have been done over the years that we really have not been able to distinguish these isomers analytically, and I think you know, the real challenge in better understanding the science of these chlorobiphenyls in the future is going to be in that area.

DR. TOTH: My name is Jason Toth with EPA. I want to direct one comment to Dr. Vodicnik. I found your data with respect to the study on the transfer

and accumulation of PCBs in offspring of maternal mouse noteworthy, if not remarkable with respect to the implications for human populations. I am wondering if the conditions under which the original deposition of PCBs in the maternal mouse occurred influenced the quantity of the PCBs that were liberated and accumulated in the mammary gland and subsequently transferred to the offspring? In other words, is this phenomena independent of the fact of the maternal mouse, for instance, being exposed, say, two, three years prior to giving birth, versus perhaps only 60 days prior to that?

DR. VODICNIK: It is awfully hard to expose a mouse three years before giving birth, but I understand your point.

Just looking at the data we have, comparing pregnant animals and virgin animals you can see that in the first two weeks there was still some redistribution of the PCB occurring. If I were to repeat that experiment now I would probably wait at least one month for redistribution of the chemical. However, when we looked at 3,4,5,3',4',5'-hexachlorobiphenyl we did study those animals for up to four months prior to mating, and what we saw there was again about one month to six weeks of redistribution and then no further elimination from tissues in virgin mice and again the same results that we saw with 2,4,5,2',-4',5'-hexachlorobiphenyl.

I think, also, the fact that there was no significant difference between virgin and pregnant animals suggests that during those early phases of pregnancy I don't think what we saw was an artifact of when the compound was administered because there were no differences at that point.

DR. TOTH: If you were to make a prediction with respect to lactating human mothers, intuitively you would be inclined to say that if they had accumulated and deposited their PCBs sometime prior to pregnancy you would not expect them to be liberating similar percentages of those quantities, would you, at all?

DR. VODICNIK: I would not expect similar percentages, and this was brought up earlier, because of the percentage of fat in rodent versus human milk, because of the species differences in dietary versus stored lipid for the synthesis of milk triglyceride, because of litter size and because of growth rate of the offspring. I would not expect those values to be anywhere near what we have seen in the mouse.

SUMMARY: Although levels of the lesser chlorinated PCBs appear to be decreasing, it is not clear that this is the case with the more stable, highly chlorinated PCBs. Because of the widespread distribution of PCBs, their presence in human tissues and their transfer from mother to infant via lactation it seems advisable to continue monitoring for PCBs and to investigate more fully possible effects of PCBs on the developing infant.

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CHAPTER 4

EXPOSURE STUDIES - INDUSTRIAL PROCESSES

Previous chapters have dealt with issues surrounding commercially-produced PCB (e.g., Aroclors). Hodges et al. present the very complex technical problems associated with the detection and quantification of trace level PCB generated as incidental products of highly diverse chemical processes. The pattern of PCB thus produced relates to the specific commercial process under investigation. Attempts at identification of these PCB by comparisons to Aroclors are of little value. Several possible solutions to this dilemma are suggested together with their technical limitations. The discussion summary elaborates on the problems of differential matrices in which PCB must be identified, the presence of many interfering substances, and the basis for the 50 ppm regulatory limit. In addition to the issues of limits of detection and quantification, is the difficulty associated with analyzing 209 potential PCB congeners whose total quantity cannot, by law, exceed 50 ppm. The paper concludes with an overview of the total incidental PCB produced per year (13,800 lbs) by 135 chemical processes, and their fate in terms of disposal or encapsulation in manufactured products.

EXPOSURE STUDIES RELATING TO INDUSTRIAL PROCESSES CONTAINING INCIDENTAL PCB

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Three topics are discussed: (1) Techniques for the estimation of PCB incidentally formed in chemical processes, (2) the potential for worker exposure, (3) the potential for environmental release and (4) the relative importance of that release, compared to existing environmental pools of PCB. The first topic is emphasized, but a Chemical Manufacturers Association survey that covers the other three topics is also summarized.

TECHNIQUES FOR THE ESTIMATION OF INCIDENTAL PCB

The following is a quote from "Recommendations for Improving the Reliability and Acceptability of Analytical Chemical Data Used for Public Purposes" by L. B. Rogers, et. al., who represent an Ad Hoc Subcommittee (of the Joint Board/Council Committee of Science of the American Chemical Society), dealing with the Scientific Aspects of Regulatory Measurement (Rogers et al. No date.)

> "Analytical chemical data are central to governmental and industrial decisions relevant to regulations that can have far-reaching impacts on society. When the results of chemical analysis are used for a regulatory purpose, the information must not only be reliable and creditable from a scientific point of view, but must also be expressed in terms that the general public can understand and accept. The demands placed on reliability and creditability are especially great because the general public is likely to encounter chemical data in a contentious situation where it has no basis for determining the true state of affairs. Analytical chemists must always emphasize to the public that the single, most important characteristic of any analytical chemical result obtained is an adequate statement of its uncertainty interval. It is critical that the public appreciate the existence and nature of uncertainty in scientific measurements."

Analytical Measurements "As Chlorinated Biphenyls" vs "As Aroclors"

The degree of uncertainty associated with PCB analysis as Aroclors has been discussed extensively in the literature over the past fifteen years. However, the uncertainty in measuring incidental PCB, or non-Aroclor resembling PCB, in chemical process streams has only recently been described. Basically, this is because the analytical science associated with incidental PCB is only 2-1/2 years old. This paper represents the first attempts by industrial analytical chemists to quantitate these species.

The focus of this paper is on the detection, identification and measurement of chlorinated biphenyls in the complex matrices of chemical production process streams, where these substances occur as contaminants measured in parts-per-million. The analytical problems in process stream analysis are significantly more complex than the problem of analyzing for Aroclor fluids and similar PCB products in environmental samples, biological fluids and transformer-oils.

One to ten chlorine atoms can be attached to a biphenyl molecule, thus creating ten chlorobiphenyl "homologs." While the term homolog is usually applied to the description of an aliphatic hydrocarbon series, its use throughout this paper denotes the homology of chlorine in the chlorobiphenyl series. Hence, when we refer to a homolog class, we will be talking about a given degree of chlorination. There are a total of 209 possible congeners among the ten homologs as shown here (Table 1). Recognition of the fact that chlorobiphenyls are actually 209 individual, identifiable, chemical compounds is important because virtually every compound has different chemical and/or physical properties. The problems in analyzing chlorobiphenyls from process streams can therefore involve the detection, identification, and quantitation of one to 209 separate chemical substances.

In the U. S., the principal manufacturer produced a specified series of these mixtures under the trade name Aroclor fluids. Each of these fluids is estimated to contain up to 60 different chlorobiphenyl congeners. Because each of these fluids was manufactured under carefully controlled conditions by the direct chlorination of biphenyl, the number, identity, and concentration of congeners in each Aroclor fluid was very consistent. When one speaks of PCB, the image generally created is that of one or more of these Aroclor fluids.

The minute amount of chlorinated biphenyls generated during routine chemical manufacturing does not produce "Aroclor" fluids. This fact is of extreme importance when considering issues of detection, identification, and quantitation. When chlorinated biphenyl occurs in a normal chemical process, it does not necessarily result from the chlorination of biphenyl. Incidental generation of chlorobiphenyls occurs through different sets of reaction mechanisms and consequently, produces a different congener mix of chlorobiphenyl substances.

These processes may produce a single congener, a series of specific isomers within a given homolog class, or a random mix of various congeners, but none are the same as the pattern produced in Aroclor fluid manufacture.

The capillary chromatogram shown in Figure 1 shows the separation of a series of trace level chlorinated biphenyls which were isolated from a chemical process stream after weeks of intensive analytical effort. The major component eluting at 31.8 minutes is equivalent to 3 ppm based on the original sample. The chromatographic profile appears to have many of the characteristics of an Aroclor fluid; however, closer observation shows that only two or three of the components have common retention indices. After GC/MS confirmation, it was learned that all 26 resolved components were isomers of the same homolog class.

The chemical manufacturing situations which generate these minute quantities of chlorinate biphenyls are extremely diverse, and the type and quantity of congeners produced are unique to each process. At least 41 different chemical reactions are known which lead to the generation of chlorobiphenyls, of which direct chlorination of biphenyl is only one. It is important to note that congeners which do not occur (or have not been observed) in the direct chlorination of biphenyl (i.e., Aroclor fluid manufacture), can and do occur, and have been observed in chlorobiphenyl incidental generation.

Table 1. Distribution Of Chlorine Among Possible PCB Congeners

		Chlorine Atoms on Ring A				
		1	2	3	4	5
		Congeners				
	0	3	6	6	3	1
Chlorine	1	6	18	18	9	3
Atoms	2		21	36	18	6
on	3			21	18	6
Ring	4				6	3
B	5	—	—	—	—	1
Total:		9 +	45 +	81 +	54 +	20 = 209

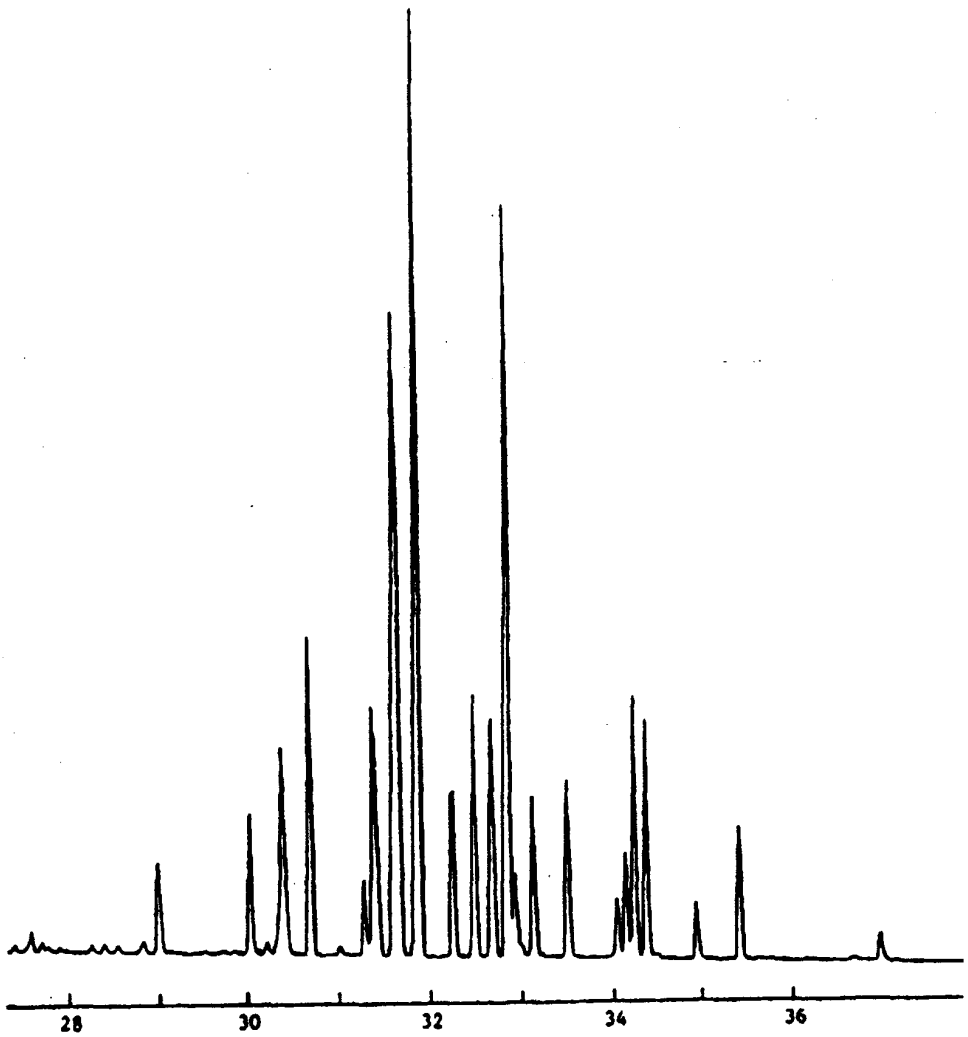


Figure 1. Capillary chromatogram of 26 PCB homolog isomers isolated from a chemical manufacturing process stream.

Status of the Identification of Chlorobiphenyl Congeners

The identification of chlorobiphenyl congeners has been the subject of extensive research. Almost all research on isomer identification has been based on analysis of Aroclor fluids. For example, Webb and McCall (1972) separated component isomers from Aroclor fluids by preparative gas chromatography and identified them by comparison with synthetic materials, using both GC retention times and infrared spectra.

Sissons and Welti (1971) characterized the major constituents of Aroclor 1254 by proton magnetic resonance and mass spectrometry after previous separations by gas chromatography.

Pellizzari et al. (1981) identified 73 PCB congeners in a mixture of Aroclor 1016, 1254 and 1260 fluids using capillary gas chromatography with electron capture detection. Confirmation was obtained using capillary gas chromatography/negative chemical ionization mass spectrometry. Albro et al. (1977, 1979, 1981) provided characterizations of Aroclor fluids using capillary gas chromatography. In Aroclor fluids 1248, 1254 and 1260, they found 110 congeners and were able to identify 106, based on method of co-chromatography (coincidence of retention indices) using 45 available standards and "best fit" matching of summed half indices. Further confirmation of identification was obtained by separating the Aroclor fluid into fractions containing four, three, or less than three ortho-substituted chlorine by absorption on charcoal, as described by Jensen and Sundstrom.

Albro et al. found that the retention behavior of chlorobiphenyl congeners in Aroclor fluids is sufficiently different for different liquid phases that one can generally find a phase capable of resolving any given isomer pair. Albro and coworkers have demonstrated a technique which holds promise for successful solution of one key problem in chlorobiphenyl analysis: The successful resolution of all isomer mixtures into their individual components and by providing a catalogue of 135 congener retention indices on a variety of columns, and by predicting the retention indices of the remaining congeners. However, Albro warns the users of this technique that further confirmation studies using IR and NMR with known standards is still necessary. This comment is particularly appropriate for those congeners which have never been identified in Aroclor fluids, but which can and do occur in process streams.

Little has been published in the literature concerning the isolation and characterization of incidentally generated chlorobiphenyls, perhaps due to the fact that incidental chlorobiphenyl congeners are present in process streams at levels below 10 ppm and, in many cases, less than 1 ppm. Aroclor characterizations, on the other hand, have dealt with congeners present at the percent level with no matrix.

The techniques required for the concentration, resolution and isolation of the 26 homolog isomers shown earlier are typical of the sophisticated work effort required to characterize incidental chlorobiphenyls in some process streams.

First, vacuum fractional distillation of kilogram quantities of process sample is required to concentrate the chlorobiphenyls. Second, the sample matrix is separated from the chlorobiphenyls by first a macro-scale absorbent column using alumina, silica gel, Florisil or a combination of these, and then after concentration, a micro-polishing cleanup is needed. High pressure liquid chromatography is then used to separate and isolate the individual chlorobiphenyl congeners for subsequent identification by infrared and nuclear magnetic resonance spectroscopy.

Individual chlorobiphenyl isomer resolution for all members of a homolog class cannot generally be obtained, even on chromatographic systems with more than 30,000 theoretical plates. Therefore, spectroscopic confirmation is often difficult in the absence of standards.

The problem of specific isomer identification has relevance to both the analysis of chlorobiphenyl in environmental samples (where the contamination may have occurred from Aroclor or similar type fluids) as well as the analysis of chemical process streams. Pattern recognition methods are based on the identification of a specific Aroclor fluid from the "pattern" of congeners observed, and then analyzing the environmental sample as if it contained such a fluid. However, it has been shown that the environment changes the pattern, that the toxicity of specific isomers varies, and that such short-cut methods do not show the presence or absence of the toxic components (Albro et al. 1979).

While the Aroclor fluids have been quite thoroughly characterized, the current state-of-the-art is not sufficiently developed to permit positive identification of the possible 209 congeners which might be present in a chemical process stream at the low ppm levels. Systematically, this can be attributed to the fact that only eighty chlorobiphenyl congeners are available commercially (Ultra Scientific, Inc.). In addition, the work effort required to separate, isolate and identify the quantities of chlorobiphenyls present in chemical process streams is orders of magnitude more complex than Aroclor characterizations.

Reasonable scientific evidence, however, is developing for approximately 135 congener identifications based on the co-chromatography approach of Albro. Succinctly stated, given the current state-of-the-art, the quantity of chlorobiphenyls cannot be determined unless the congeners present can be identified, and a known standard for each congener exists to calibrate the instrument.

Analytical Methods for Chlorobiphenyl Detection, Identification and Quantitation are Limited

All applicable methods are based on some form of instrumental analysis. Instrumental methods capable of detecting chlorobiphenyls are also applicable to their quantitative measurement with varying degrees of precision and accuracy.

Current known instrumental methods with some degree of applicability to detection of chlorobiphenyls are:

Ultraviolet Spectroscopy;
Infrared Spectroscopy;
Nuclear Magnetic Resonance Spectroscopy;
High Pressure Liquid Chromatography;
Gas Chromatography; and
Mass Spectrometry.

Ultraviolet, infrared, and nuclear magnetic resonance spectroscopy lack the selectivity and sensitivity to measure chlorobiphenyl in the parts-per-million range of interest in most chemical process streams. Some studies have been made on high pressure liquid chromatography methods, but the primary limitation is the lack of a detector which is selective, and nondiscriminating for chlorobiphenyls. Only gas chromatography and mass spectrometry have demonstrated sufficient selectivity and detection sensitivity to be applicable to chlorobiphenyl measurement in ppm concentration ranges.

Analytical method development for chlorineted biphenyl analysis must be cognizant of criteria other than the best suited instrumental technique.

Analytical methods have two types of characteristics (Bandal et al. 1981) - scientific and practical. The scientific characteristics determine the relisbility of the analytical data; the practical characteristics determine the utility of the method. The scientific attributes of a method include such things as accuracy, precision, specificity, and limit of reliable measurement; the practical attributes include cost of performance, time required, and level or training needed. For research purposes, the practical aspects are of secondary consideration; for regulatory operations of compliance and surveillance, practicality is of great importance. Little enforcement is possible using a method which turns out one analytical value per day!

This is one area where industrial analytical chemistry and regulatory surveillance has a strong common interest. Industry demands rapid, accurate analytical numbers to control processes and approve shipments of product. Regulatory surveillance is considerably more reliable and effective if the data can be generated using a simple, rapid analytical method.

Quantitative Analysis of Chlorobiphenyls in Process Streams (LOD and LOQ)

Given the detection and identification of one or more chlorobiphenyl isomers, one can then proceed to measure the amount present. Instrumental methods applicable to detection are generally applicable to quantitation of the substance, except that a new series of technical problems is then encountered. Quantitation must deal with the limit of detection (LOD) of the detector, changes in the sensitivity of the detection mechanism to changes in concentration of a chlorobiphenyl congener (linearity), variation in detector response between one chlorobiphenyl and another (discrimination), variation in the analysis as performed from day to day, (precision) as well as the influence of the sample matrix on detector response interference, recovery).

Certain terms, Limit of Detection (LOD) and Limit of Quantitation (LOQ), need to be defined in order to establish a common basis for discussion of these issues of detectability and quantitation.

Each detector generates a background electrical output signal which is the sum of electronic and chemical noise. This background signal is not constant, but varies slightly over time. The electrical signal generated by a substance to be measured must be greater than this variation in background signal by a specified amount in order to be sure that what is observed is truly a measurement of a substance and not a random variation in the background signal. The LOD is therefore defined as a signal which is at least three times the background signal-to-noise ratio.

The second term to be defined is the LOQ. The LOQ is defined as the quantity of a substance which must be presented to a detector in the presence of a sample matrix to achieve a signal which is ten times the detector signal-to-noise ratio. This concentration represents an amount which can be reliably measured and reproduced if the samples were analysed by many different laboratories. The LOQ definition states that signals less than this magnitude cannot reliably be used to measure the quantity of a substance, much less regulate it. The importance of these terms cannot be overemphasized in establishing not only creditable analytical methods, but also creditable regulatory limits.

Let's examine for a minute what industrial analytical chemists have been trying to attain in terms of LOQ over the past 2-1/2 years to ensure regulatory compliance at 50 ppm total incidental PCB. We must measure the presence of 209 individual chemical species and determine if the sum of these measurements is less than 50 ppm. The LOQ therefore for each congener must be 50 ppm divided by the potential 209 congeners or 0.24 ppm per congener. The limit of detection then must be 0.24 ppm divided by 3.33 or 72 ppb per congener to have a creditable analytical method.

This is presently unattainable. In the past, socio-political and/or toxicological considerations have sometimes led to a perceived need to have regulatory limits that are less than the current LOD. This occurs when exceedingly small concentrations are considered to be dangerous, constituting considerable risk to human health.

In such a situation, the regulatory limit is usually set at the detection limit, as has been done as an out-growth of the well-known Delaney amendment. However, there are powerful scientific reasons for setting a regulatory limit not only above zero, but also "reasonably" far above the detection limit. First, it is impossible to prove scientifically the absence of any given chemical species. The best that can be accomplished by the measurement process is to establish a limit below which the analytical error is unacceptably large. Second, apart from the measurement limitation, it is impossible in principle to achieve a state of complete purity for any sample. Third, it is extremely difficult to make reliable measurements, and especially so at the trace level. Fourth, one should note that analytical methods have been improving (and will undoubtedly continue to improve) in selectivity and in reaching lower limits of detection so that a concentration that is below the limit today may be above it tomorrow. Hence, when the regulatory limit is set equal to, or just above, a current limit of detection, the timing of the analyses relative to that of regulatory enforcement introduces yet another uncertainty. Therefore, one must recognize the compelling scientific reasons to establish a regulatory limit above the detection limit and to define

acceptable analytical risks (i.e., the documentation of false positive/negatives, as well as "statistical" errors).

Electron Capture Detectors

Although gas chromatography in conjunction with electron capture detection is the most widely accepted technique in use today for the determination of polychlorinated biphenyls, it is a technique which is more applicable to the determination of Aroclor fluid type materials than to those chlorobiphenyls which are likely to form in various types of chemical processes. The predictable isomer patterns of the different Aroclor fluids or their mixtures makes recognition of their presence in a sample relatively easy. In addition, the various Aroclor fluids are still readily available for use as standards and detectable concentrations as low as 2 µ/g/L (or approximately 2 ppb) have been reported (Delfino and Easty 1979, Williams and Benoit 1979) for such diverse samples as paper mill effluents and household products. Lowest detectable concentrations in samples such as transformer oils (Ogata et al. 1977) appear to be 1 ppm.

Besides being sensitive to chlorobiphenyls, the electron capture detector is also sensitive to chloronaphthalenes, chloroterphenyls and chlorophenylethers as well as sulfur compounds, all of which may elute with the various Aroclor groups (Kapila and Ave 1977, Armour and Burke 1971). While this presents problems in the analysis of samples for Aroclor compounds, it is a much more serious problem in the analysis of chemical process stream samples for chlorobiphenyls since those formed in any given chemical process do not exhibit the pattern of isomer distribution profiles shown by the various Aroclor fluids.

The capillary chromatogram at the far left of Figure 2 represents the equivalent of 1 ng of Aroclor 1242 in hexane which, relative to a sample preparation scheme, could equate to 50 ppm. The identification, if this were a sample extract, would be unequivocal. The middle chromatogram represents three ng's, 1 ng each, of the three monochlorobiphenyl isomers, and the chromatogram at the far right represents 1 ng or the equivalent of 50 ppm, of 3 chlorobiphenyl. This effect of losing the chromatographic profile of PCB makes chlorobiphenyl identification and quantitation very difficult using electron capture detection and consequently, the limits of detection for incidental PCB measurement are two or three orders of magnitude higher.

In addition, there is a high probability that nonchlorobiphenyl components in a process stream sample would elute within the chlorobiphenyl retention time region. This makes it virtually impossible to say, with any degree of certainty, which chromatographic peaks represent real chlorobiphenyls and which represent nonchlorobiphenyl interferences.

The sensitivity and LOD of the electron capture detector varies over a tremendous range from homolog to homolog and from isomer to isomer within homolog. The range of the variability is illustrated in Table 2 from Hutzinger's book, "The Chemistry of PCB's."

Note that the molar response of decachlorobiphenyl is 7,000 times greater than the response to the same molar concentration of 3-chlorobiphenyl. Note

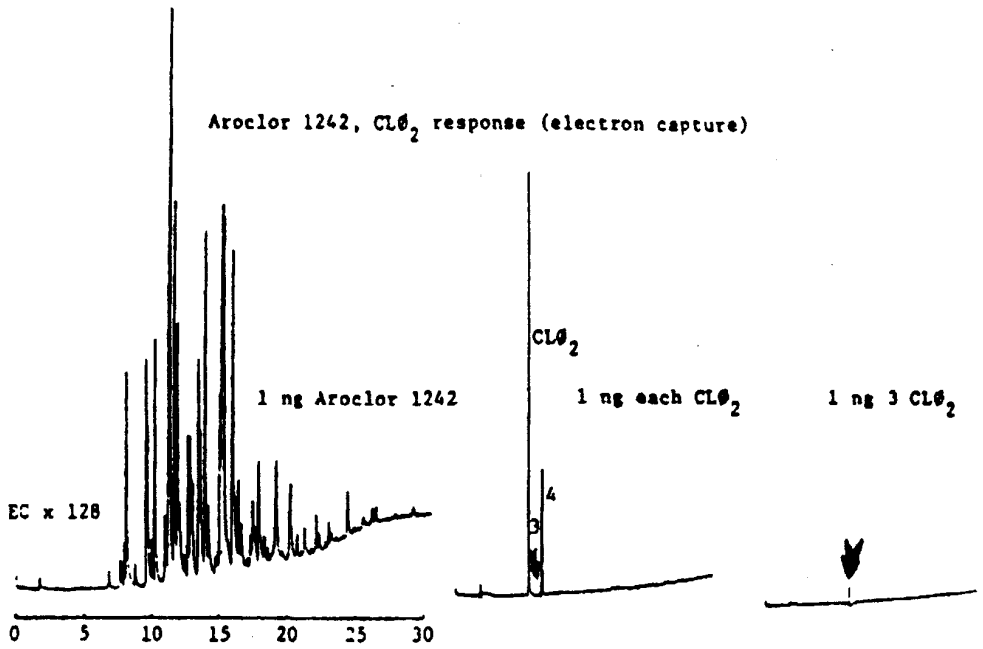


Figure 2. Electron capture response of monochlorobiphenyls vs. Aroclor 1242.

Table 2. Relative Molar Responses of Electron-Capture Detectors to Some Chlorobiphenyls

	Relative Molar Response
2-	1.00
3-	0.20
4-	1.10
2,2'-Di	5.16
2,4-Di	17.7
2,6-Di	32.0
3,3'-Di	6.10
3,4-Di	15.2
4,4'-Di	5.97
2,4,4'-Tri	135
2,2',4,4'-Tetra	106
2,2',6,6'-Tetra	20.6
3,3',4,4'-Tetra	396
3,3',5,5'-Tetra	320
2,3,4,5-Tetra	367
2,3,5,6-Tetra	259
2,2',4,4',6,6'-Hexa	347
3,3',4,4',5,5'-Hexa	726
2,2',3,3',4,4',6,6'-Octa	1,180
2,2'3,3',5,5',6,6'-Octa	1,150
Deca	1,410

also the difference in response between the isomers of the tetrachloro homolog, which vary by a factor of 20 for the same molar quantity of chlorobiphenyl. The full range of this variation is not known since the absence of standards has prevented research on the issues. The importance of the variation in electron capture response must be clearly understood. For incidental PCB quantitation, each congener in the process sample must be completely characterized, and a standard of the characterized congener must be available in suitable purity for calibration either by purchase or by synthesis. In addition, the chlorinated biphenyl congener must be completely separated chromatographically before being presented to the detector or coeluting isomers may generate large false positives or negatives.

At the present time, only 80 of the possible 209 chlorobiphenyl isomers are commercially available, (Ultra Scientific, Inc.) making the calibration procedure almost hopeless because of the diversity of response among the individual isomers with this detector. This factor, together with the coelution of unknown interferences, acts to preclude the use of the electron capture detector for the quantitative analysis of process and waste stream samples for incidental generated chlorobiphenyls.

In some specific instances, it may be possible to calibrate an electron capture detector-based analysis procedure with a mass spectrometer for a specific process stream. However, it can be expected that both the number and amount of chlorobiphenyl isomers in a process stream can change as process conditions vary over time, thus calling into question the accuracy of the calibration performed. Frequent recalibration with a mass spectrometer would be necessary.

Finally, one additional factor makes use of the electron capture detector difficult in most process stream analyses. The detector response is extremely sensitive to chlorine containing compounds, which is the basis for is utility in chlorobiphenyl analysis. However, when chlorobiphenyl is formed in process streams, the matrix is usually organo-chlorine compounds. Since the chlorobiphenyl is present in ppm levels, a suitable sample cleanup is generally needed.

Perchlorination as a Chlorobiphenyl Analysis Sample Preparation Procedure

Since chlorobiphenyl mixtures contain many chlorobiphenyl isomers which are difficult to identify and analyze, a procedure called perchlorination was developed to analyze low concentrations of Aroclor fluids. The perchlorination procedure reacts the lower chlorinated homologs and isomers with antimony pentachloride, $SbCl_5$, to form the single compound decachlorobiphenyl (DCB) (Berg et al. 1972). The amount of DCB so formed is then determined by gas chromatography. This procedure was developed as a means of lowering the analytical LOD when analyzing for trace quantities of Aroclor fluids in environmental samples. In these instances, the typical Aroclor fluid gas chromatographic pattern may be recognizable, but the concentration is too low for accurate quantitation. Conversion of all the chlorobiphenyl isomers into DCB permits the measurement of a single peak and the use of a known high purity and readily available standard. Perchlorination does not, however, yield any data regarding the concentration or distribution of specific chlorobiphenyl isomers.

In applying the perchlorination reaction to the analysis of Aroclor fluids, various researchers observed that the method could yield erroneous results (Armour 1973, Stratton et al. 1979, Trotter and Young 1975). While the specified reaction conditions yielded 90-100 percent conversions of Aroclor 1254 to DCB, conversions of only 30-70 percent were obtained for Aroclor 1242. Higher reaction temperatures resulted in poor recovery of the mono- and di- substituted isomers due to the apparent instability of these species at the higher temperature.

Perchlorination techniques in general are not applicable for chlorobiphenyls in process streams due to serious interferences which generate false positives.

Positive interference in the perchlorination procedures arises from the presence of biphenyl, naphthalene or chlorinated naphthalene in the sample. Process streams can contain these and other potential interfering organic compounds. Biphenyl and naphthalene are for example, heat transfer agents used in industrial heat exchange equipment, and are, therefore, a potential contaminant in process streams (Tindall and Wininger 1980, Burkhard and Armstrong 1981).

In addition, polychlorobenzenes present in some process streams can condense in the presence of antimony pentachloride to make chlorobiphenyls which in turn would be chlorinated, resulting in false positives.

Electroconductivity Detector

The electroconductivity (Hall) detector operating in the chloride mode, has also been used in the gas chromatographic analysis of chlorobiphenyls, but to a far lesser extent (Sawyer 1978a,b, Mindrup 1979). In contrast to normal operation, the detector furnace must be at temperatures above 820°C (Dolan et al. 1972) or even as high as 1100°C (PPG Ind., Inc.) to effect the pyrolysis of chlorinated biphenyls. The detector responds specifically to chlorine containing compounds and has a greater degree of selectivity for chlorobiphenyl analysis than the electron capture detector. In addition, the response is a direct function of the halogen content of the compound and is linear. However, the sensitivity in terms of molar response at these elevated detector temperatures is at least one and possibly two orders of magnitude lower than the electron capture detector. Suitable sample pretreatment in terms of cleanup must be incorporated when this detector is applied to process stream analysis.

Gas Chromatography/Mass Spectrometry in Quantitative Chlorobiphenyl Analysis

As noted before, gas chromatography combined with either electron capture or electroconductivity detectors are of little value for the quantitation of trace quantities of uncharacterized chlorinated biphenyls. In both cases, they require standardization with known compounds or by a principle analytical method such as gas chromatography/mass spectrometry. GC/MS can be used to identify and quantify individual chlorobiphenyl isomers in a process stream. However, the identification is limited to (1) confirmation that an eluting substance is a chlorobiphenyl, and (2) elucidation of the chlorobiphenyl homologue class. In most cases, the specific isomer cannot be identified.

While total scans from 100-600 atomic mass units are desirable for confirmation during the initial sample screening, Selected Ion Monitoring (SIM), also known as Single Ion Monitoring or Multiple Ion Detection is the technique most frequently employed for quantitation, and offers at least an order of magnitude higher sensitivity than total-scan (Eichelberger et al. 1974). Two compromise techniques between total scan and SIM have also been described: (Canada and Regnier 1976) limited mass scanning and mass chromatography. Both approaches represent modification in computer data collection rates for ion intensity measurements, so chlorobiphenyl confirmation or selectivity will not be sacrificed in achieving high sensitivity.

In spite of a higher limit of detection as compared to the electron capture detector, SIM is the most specific method for the identification of a substance as being a chlorobiphenyl and for the quantitation of chlorobiphenyls of unknown congener and homolog distribution. The technique essentially involves using the mass spectrometer to measure the integrated ion intensitive of ions characteristic of the species of interest. For the chlorobiphenyls, the ions are typically the molecular weight ions resulting from the naturally occurring chlorine isotope abundance ratios M and M+2. (Table 3).

Thus, for mono through heptachlorobiphenyl, the following mass ions are monitored. For octa and nonachlor, the M+2 and M+4 mass ions are the most intense, and for the decachlorobiphenyl, the M+4 and M+6 ions are used. The intensity ratios for each homolog are used to confirm the eluting component as a chlorobiphenyl.

The ions for each homolog can be monitored in groups spanning the retention time windows of the various homologs which are established by standards. The qualitative criteria for confirmation of eluting chlorinated biphenyls have been described by two authors, Tindall and Wininger, (1980) Collard and Irwin (1982) independently. They are: the chromatographic peak of the characteristic mass ions must maximize at the same retention time; the peak must be in the proper retention time window; and thirdly, the relative peak intensities of the molecular ions must be within $\pm$15% of the theoretical. (Figure 3)

In essence, twenty specific ion chromatograms are generated in one 20 minute temperature programmed run. This yields a high degree of specificity. In addition, all of the chlorinated biphenyls form stable molecular ions, and the isotopic abundance ratios are fixed by nature.

Martelli (1981) and coworkers have evaluated the analytical response of 45 single chlorinated biphenyls using SIM and have found the following variability in response within the homolog groups. (Table 4) Their study documents mass spectrometry as the least discriminating detector available for use with gas chromatography, and as a result, a single chlorinated biphenyl isomer can be used to estimate the quantity of all other isomers within its homolog class.

Collard and Irwin evaluated the relative response of each homolog class as a function of concentration. The response was linear in all cases, but sensitivity decreased by a factor of ten when comparing mono to decachloro-

Table 3. Specific Mass Ions with Relative Intensities Used for Determining Chlorinated Biphenyls

Clx O_2	M (Intensity)	M + 2 (Intensity)	M + 4 (Intensity)	M + 6 (Intensity)
Mono-Cl_1	188 (100%)	190 (33%)		
Di-Cl_2	222 (100%)	224 (65%)		
Tri-Cl_3	256 (100%)	258 (98%)		
Tetra-Cl_4	290 (77%)	292 (100%)		
Penta-Cl_5	324 (61%)	325 (100%)		
Hexa-Cl_6	358 (51%)	360 (100%)		
Hepta-Cl_7	392 (44%)	394 (100%)		
Octa-Cl_8		428 (88%)	430 (100%)	
Nona-Cl_9		462 (77%)	464 (100%)	
Deca-Cl_{10}			498 (100%)	500 (87%)

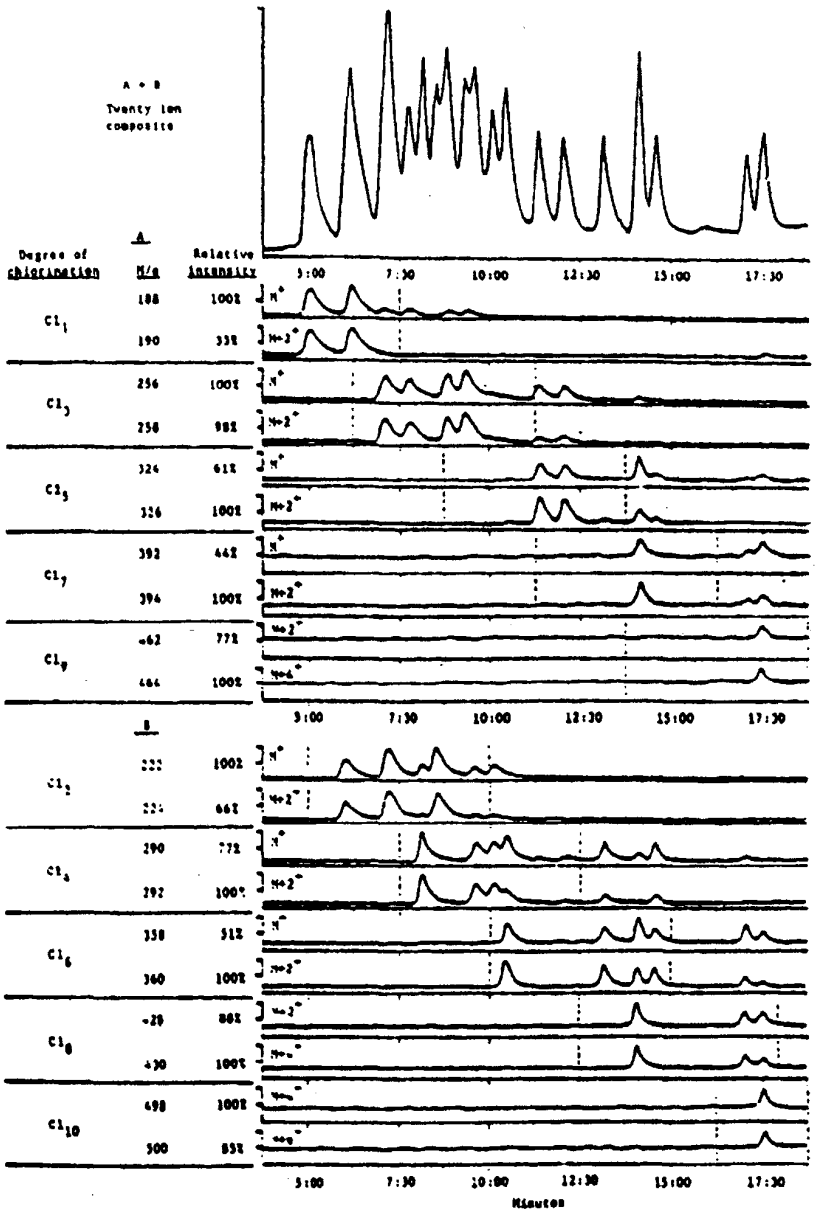


Figure 3. Composite and individual mass ion chromotograms of several chlorinated biphenyl isomers.

Table 4. Molecular Ion Response of Chlorinated Biphenyls to Electron Impact Mass Spectrometry[a]

Homolog	No. of possible structures	No. of analyzed structures	Average response	Response variability
Mono	3	3	1.050	4.4%
Di	12	10	1.736	17.2
Tri	24	9	1.334	12.4
Tetra	42	11	1.577	20.7
Penta	46	3	1.005	0.7
Hexa	42	7	1.153	10.8

[a]Martelli, GP, Castelli, MG, Fanelli, R. 1981. Biomedical Mass Spectrometry, 8, 8, 347-350.

biphenyl. (Figure 4) As shown in Table 5, recoveries performed in replicate at the two, ten and thirty ng levels had a range of 95 to 103%. Table 6 presents a precision study on ten replicate weighings demonstrated a relative error of ±20% at the 95% confidence level. The limit of detection in the complex chlorinated hydrocarbon matrix was observed as 1.0 ng of a single isomer injected on-column which was equivalent to 5 ppm per isomer in the original sample. Tindall and Wininger have reported comparable sensitivities in the absence of a matrix.

GS/MS/SIM eliminates many interferences which occur with electron capture or electroconductivity detectors insofar as only compounds which have common mass fragments and elute in the retention time window are potential interferents. The technique has a special utility in cases of incidental generation in chemical processes since it does not depend upon operator "pattern recognition" to identify the chlorobiphenyls. Even though SIM does not detect as small amounts as does electron capture or electroconductivity/gas chromatography, the increase in signal-to-noise (S/N) ratio resulting from the increased specificity offsets the decrease in absolute signal strength when analyzing process streams for chlorobiphenyls.

Sample Preparation and Clean-up Techniques

Sample preparation schemes for the analysis of chlorobiphenyls in chemical process streams often require considerable development effort. Many materials are not amenable to direct chromatographic analysis. Crystalline solids, polymeric materials, tars, and corrosive liquids are examples. Samples such as these are generally dissolved in a suitable solvent and analyzed directly after appropriate sample cleanup or extraction of the chlorobiphenyls into a more suitable solvent for chromatographic analysis. Direct extraction of solids with solvents has been used for pigments, biological samples and other materials, but liquid-solid extractions are often nonquantitative for encapsulated chlorobiphenyls, except in cases of simple adsorption of the chlorobiphenyls on the solid surface.

The problem of extracting the chlorobiphenyls from the matrix can constitute the major analytical problem in determining chlorobiphenyls since many of the matrices are similar to the chlorobiphenyls in physical, chemical and chromatographic properties. Many methods are employed to remove interferences from the sample matrix. Solvent extraction or partitioning with strong acid, usually sulfuric, is successful when the interferents are chemically dissimilar to the chlorobiphenyls, but can lead to problems with recovery of the lower chlorinated homologs (Dry Color Manufacturers Association, Lincer 1973).

Figure 5 shows the effect of a sulfuric acid cleanup on monochlorobiphenyls. A hexane solution containing 10 ppm mono and 1000 ppm Aroclor 1242 was separated by capillary gas chromatography with flame ionization detection. After shaking the solution for three hours with concentrated sulfuric acid, the monochlorobiphenyls have decreased significantly while the Aroclor fluid remains unchanged. After eighteen hours, the monos are completely absent with only minor changes in the Aroclor composition (Figure 6). This represents an MCB destruction rate of approximately 20% per hour at room temperature.

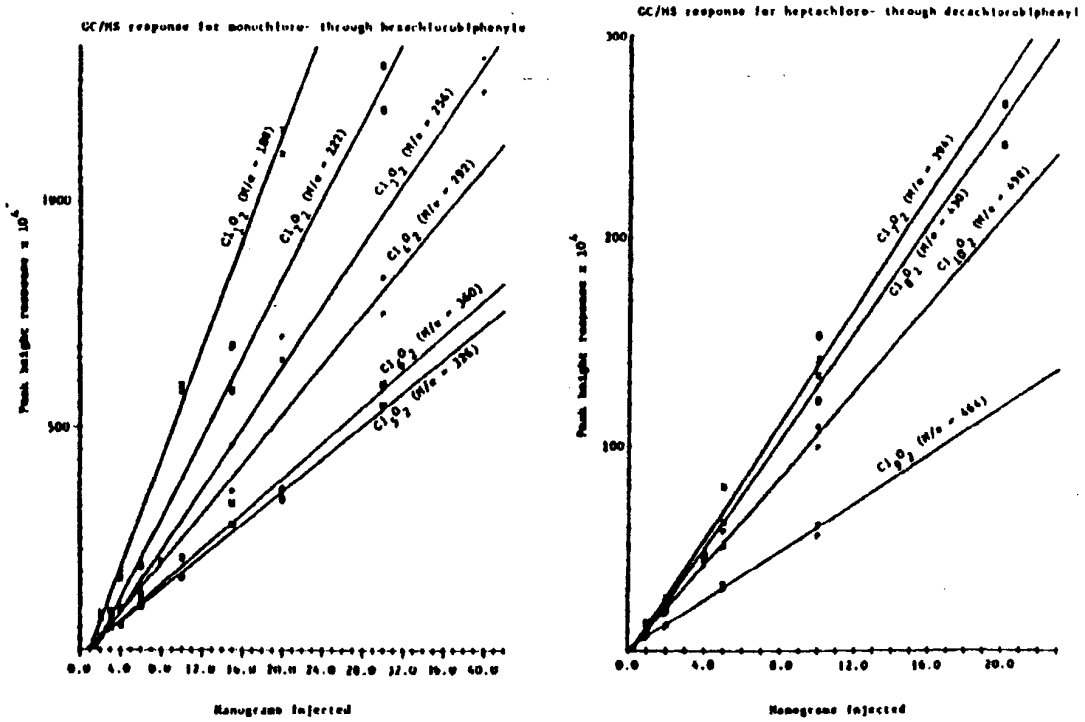


Figure 4. Linearity of response for selected mono-decachlorobiphenyl congeners using GC/MS/SIM.

Table 5. Chlorinated Biphenyl Recoveries from Spiked Chlorinated Hydrocarbon Matrix

Degree of chlorination	Run #1 Amount added (ng)	Run #1 Amount found $\bar{x}$,(ng)[a]	Run #1 Std. dev. (ng)	Run #2 Amount added (ng)	Run #2 Amount found $\bar{x}$,(ng)[b]	Run #2 Std. dev. (ng)	Run #3 Amount added (ng)	Run #3 Amount found $\bar{x}$,(ng)[c]	Run #3 Std. dev. (ng)	Mean percent recovery
1	2.0	1.9	0.05	10.0	9.7	0.2	20.0	19.0	1.1	96
2	3.0	3.0	0.08	15.0	14.8	0.4	30.0	28.1	1.3	98
3	4.0	4.1	0.09	20.0	19.7	0.7	40.0	38.1	1.6	99
4	3.0	2.8	0.21	15.0	15.3	0.9	30.0	27.9	0.8	96
5	2.0	1.8	0.12	10.0	10.0	0.3	20.0	18.6	0.8	95
6	3.0	2.8	0.15	15.0	15.2	0.6	30.0	28.9	1.0	97
7	1.0	1.0	0.15	5.0	5.2	0.2	20.0	9.8	0.3	101
8	2.0	2.0	0.08	10.0	10.3	0.2	20.0	20.0	0.2	100
9	1.0	1.0	0.06	5.0	5.1	0.3	10.0	10.1	0.2	99
10	1.0	0.9	0.10	5.0	5.0	0.3	10.0	9.7	0.1	96

[a]Mean value of 5 determinations.
[b]Mean value of 3 determinations.
[c]Mean value of 2 determinations.

Table 6. Relative Precision of Chlorinated Biphenyl Measurements (Sample 2)

Degree of chlorination	Nanograms Run number 1	2	3	4	5	6	7	8	9	10	Mean (x)	Relative Precision (95%)
1	6.8	6.6	7.2	6.9	7.0	7.5	7.6	8.3	7.3	7.7	7.3	14%
2	37.3	36.3	38.0	37.4	35.9	31.4	32.2	34.1	31.0	32.3	34.6	16%
3	45.5	47.7	46.1	46.2	45.8	39.5	39.0	42.8	39.4	41.8	43.4	15%
4	24.8	25.4	25.6	25.9	25.7	21.2	21.1	23.3	21.3	23.2	23.8	17%
5	12.8	12.4	12.6	13.4	13.0	10.8	10.8	11.7	10.7	11.1	11.9	18%
6	9.4	9.4	9.6	9.6	9.8	7.8	7.9	8.1	7.8	8.3	8.8	19%
7	6.0	6.0	5.9	5.9	6.4	4.5	4.9	4.9	4.8	4.8	5.4	25%
8	4.6	5.3	5.2	5.5	5.4	4.3	4.3	4.6	4.6	4.6	4.8	19%
9	5.3	5.7	5.8	5.9	5.8	4.3	4.4	4.8	4.7	4.9	5.2	24%
10	5.7	5.9	5.8	6.0	6.0	4.9	5.1	5.7	5.1	5.5	5.6	14%

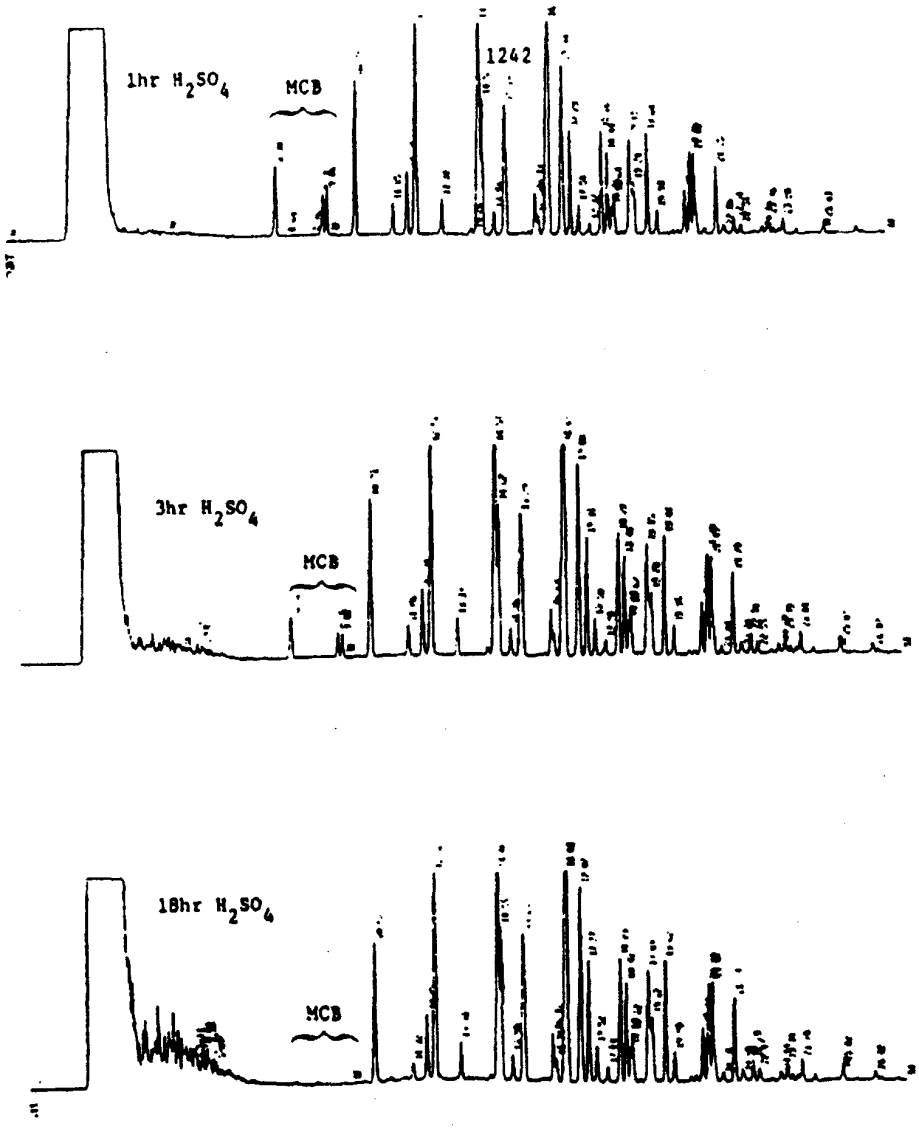


Figure 5: Monochlorobiphenyl stability in the presence of CONC sulfuric acid.

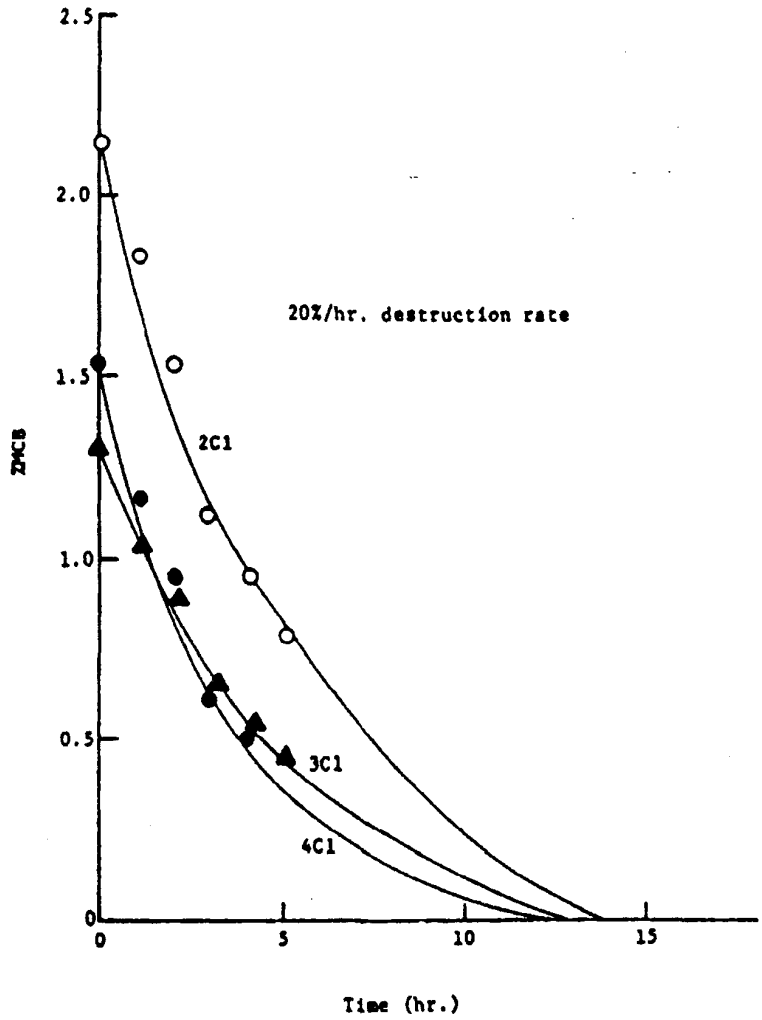


Figure 6 MCB Destruction With H_2SO_4 (CON) at 35°C.

In many cases, column chromatography is required to remove interferences. Alumina and Florisil are commonly used as adsorbents. The development of cleanup procedures using column chromatography are laborious, time consuming, and often, add considerable variability to the analysis. Maintaining a constant activity for the adsorbent and reproducing it is extremely difficult (Hutzinger et al, 1974) and is often the cause of poor interlaboratory reproducibility. In addition, the selectivity and loading capacity of a given sorbent separation must be meticulously evaluated for the lower chlorinated homologs as well as the higher ones. This has not been the case with many of the cleanup techniques which have been described in the literature for Aroclor quantitation. Figure 7 shows the eluting characteristics of some of the lower chlorinated homologs on basic chromatographic alumina using hexane as the eluant.

The ratio between chlorobiphenyl congener concentration and weight of alumina is 500/1 (alumina/congener), so sorbent capacity is not exceeded.

This cleanup technique as described in the literature states that all PCB elute in the first 5-6 bed volumes, where in fact, only 50% of the 2,6-dichloro, 25% of the 2,4-dichloro and none of the 3- and 4-chlorobiphenyl have eluted. These two areas of sample pretreatment, mineral acid digestion and adsorbent column cleanup, represent areas of published PCB analytical methodology which must be reevaluated before they can be applied to incidental chlorinated biphenyl analysis.

A multidimensional chromatographic technique utilizing both high performance preparative scale liquid chromatography and capillary column gas chromatography has been developed recently for the determination of polychlorinated biphenyls in transformer coolant and motor oils by Chesler et al. (1982). While this technique is not directly applicable to the determination of chlorobiphenyls in process streams, it affords a higher degree of resolution than the conventional gravity flow adsorbent cleanup techniques without sacrificing column capacity. In addition, such systems should be much faster cleanups and readily lend themselves to automation.

The final cleanup to remove sample matrix interferences in almost all chlorobiphenyl analysis schemes is the selection of the appropriate gas chromatographic column. Often the type of complexity of the matrix will dictate whether packed column or capillary columns should be employed. The efficiency or separating power of capillary columns is sometimes not as desirable as the selectivity which can be obtained by polar phases or mixed phases using isomer selective modifiers such as the Bentonite clays. Three and four chlorobiphenyl are extremely difficult isomers to separate on most conventional packed columns and only slightly better on the bonded SE-30 and SE-54 methyl silicone fused silica capillary columns.

Their resolution is imperative when using an electron capture detector since the 4-chlorobiphenyl isomer is 6 times more responsive than the 3-chloro isomer. Binary liquid phase optimization such as described by Laub and Purnell (1976) however, allow for the systematic design of separations to resolve isomers as well as matrix interferences.

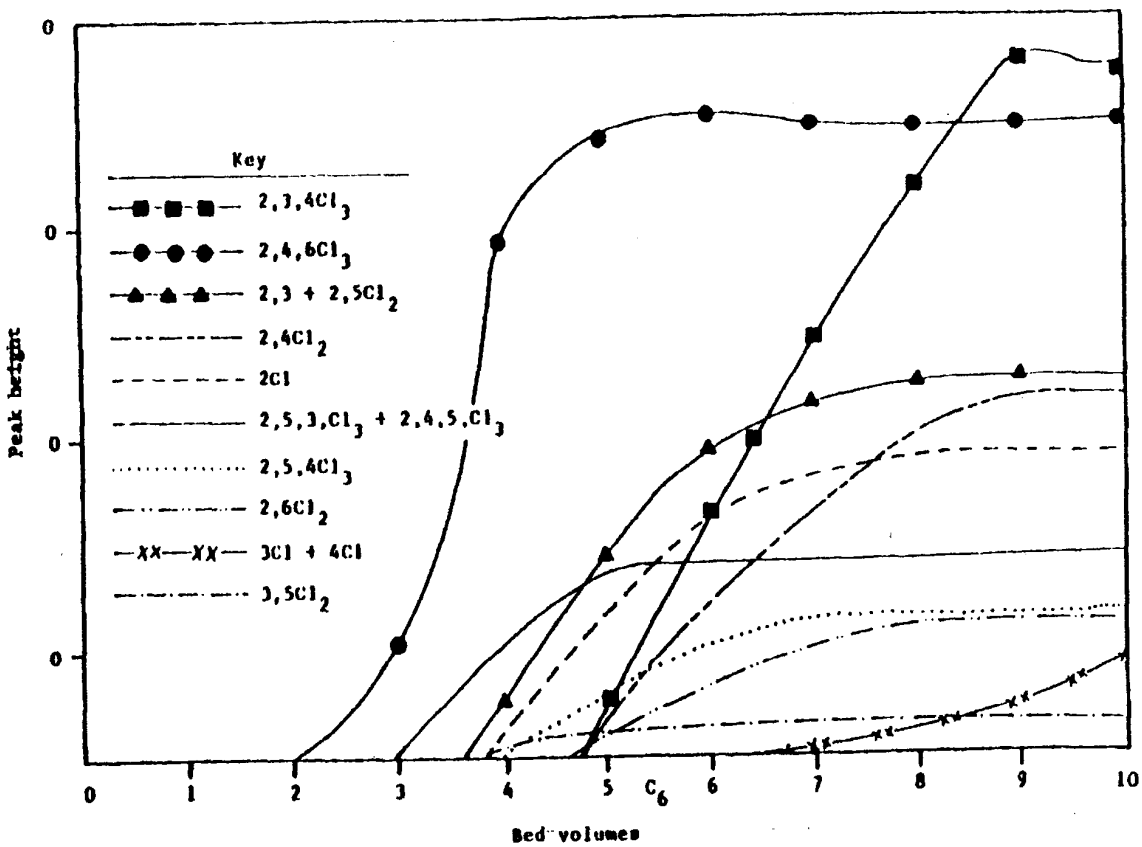


Figure 7. Lower chlorinated biphenyl elution characteristics from alumina with hexane.

Packed or capillary column selection for the analysis of PCBs as Aroclors is really academic since the pattern recognition can easily be accomplished using either separation mode. For incidental chlorobiphenyl analysis, the trend obviously needs to be towards more resolving power (capillary columns) because of the lower LODs required per eluting congener. This trend will become more apparent as good fused silica capillary columns with polar bonded phases become available.

Quality Assurance in Qualitative and Quantitative Analysis

Quality assurance is an essential but often neglected aspect of chlorobiphenyl analysis (MacDougall and Crummett 1980). The first aspect of a quality assurance program is the development and consistant use of a fully validated method. Validation of a method requires a study of the following characteristics. First, the linearity of response for the analyte in question should be evaluated over the desired quantitation range. Second, sufficient replicates of samples which had previously been spiked should be analyzed to determine the recovery and accuracy of the method over the desired quantitation range. This includes adding the analyte to the sample matrix before any sample preparation or cleanup is performed. Thirdly, a precision study on a minimum of ten replicate weighings analyzed on two different days should be performed. Finally, any known interferences should be documented as to their effect on the quantitation of the desired analyte by running sufficient sample blanks.

Once a validated method has been established, consistant use policy, in addition to an on-going quality assurance program is important. This is necessary to document that the procedure is performing at the previously determined level. Validated analytical methodology and active quality assurance programs are prerequisites to generating creditable analytical data.

For the analysis of chlorobiphenyls in process streams, a single analytical method has yet to be agreed upon, however, gas chromatography/mass spectrometry with suitable sample cleanup appears to be the only viable alternative considering the great variability of sample matrices encountered in chemical process streams.

The Degree of Uncertainty Associated with Incidental PCB Analysis - A Round Robin

To evaluate the uncertainty in the quantitation of incidentally generated chlorobiphenyls, a recent round robin analysis of five samples was sponsored by the Chemical Manufacturers Association (1982). The samples were chosen to typify process stream matrices that are presently regulated by the incidental PCB Manufacturing Rule. The following participants were asked to generate their best effort using analytical methodology they have developed for the measurement of non-Aroclor PCBs:

Environmental Monitoring and Support Laboratory
Office of Research and Development
Environmental Protection Agency
Cincinnati, Ohio

E.I. DuPont DeNemours & Company
Wilmington, Delaware

PPG Industries
Barberton, Ohio

Surveillance and Analysis Division
Region 7
Environmental Protection Agency
Kansas City, Kansas

Dow Chemical Company
Midland, Michigan

Monsanto Company
St. Louis, Missouri

Stauffer Chemical Company
Dobbs Ferry, North Carolina

Vulcan Materials Company
Wichita, Kansas

This effort was designed to document the present state-of-the-art for chlorobiphenyl analysis when a company or independent lab is contracted to analyze sample for regulatory compliance. Each sample was described as containing one or more chlorobiphenyl congeners ranging from mono to decachlorobiphenyl.

Total congener concentrations were described as ranging from 5 ppm to less than 500 ppm in the following matrices:

A - Chlorinated Benzene Waste
B - Mixture of Chlorinated Benzenes
C - Mixture of Chlorinated Benzenes
D - Chlorinated Aliphatic Waste Stream
E - Chlorinated Aromatic Sample

Each sample was to be analyzed in triplicate with separate portions of the sample being taken for each replicate analysis.

The instrumental techniques and methods of calibration were utilized for the analysis of the five round robin samples are shown in Table 7. Eight of the ten laboratories elected to use GC/MS.

Seven of these eight labs used packed columns while one utilized an SE-30 fused silica capillary column. One of the labs utilized packed column GC with electron capture detection, while another used packed column/flame ionization detection. The flame ionization detector data was deleted from the composite summary for statistical reasons.

Summary of the data across all nine laboratories is shown in Table 8. For sample A, the mean concentration was 283 ppm. The range of the reported mean concentrations was from a low of 104 ppm to a high of 413 ppm for a length of 309. The average coefficient of variation among replicates within laboratories was $\pm$11.4% relative. The coefficient of variation among laboratories was $\pm$32% and the expected coefficient of variation for a sample sent to a random laboratory with a single replicate concentration reported would be expected to be $\pm$34%.

For sample B, the range was 70 ppm, with a coefficient of variation of 92% for a single replicate. For C, the range was 105 ppm, with a coefficient of variation of 46%. For D, the range was 261 ppm, and 72% and for E, the values were 12.9 ppm with 53% as the coefficient of variation for a single replicate.

Table 7. Round Robin Methodology for Incidental PCB Analysis

Laboratory	Technique	Calibration
A	GC/EC	18 Congeners
B	GC/MS	24 Congeners
C	GC/MS (Semiquantitative Single Replicate)	10 Congeners (1/Homolog)
D	GC/FID	10 Congeners (1 Homolog)
E	GC/MS	25 Congeners
F	GC/MS	Aroclor Mix Plus Mono
G	GC/MS	20 Congeners
H	GC/MS	10 Congeners (Internal Std)
I	Capillary GC/MS	10 Congeners
J	GC/MS	24 Congeners

Table 8. Summary of Experimental Results for all Homologs Combined for a Round Robin Experiment Across Nine Laboratories

Statistic	A	B	C	D	E
Mean	283	24	64	130	9.2
Range					
Endpoint	104 - 413	1 - 71	7 - 112	33 - 294	0.3 - 13.2
Length	309	70	105	261	12.9
C_A	11.40%	15%	8%	17%	28%
C_B	32%	85%	45%	64%	44%
C_C	34%	92%	46%	72%	53%

Comments

Mean = Mean PCB concentration across all laboratories
Range = Range of PCB mean concentrations across all laboratories
C_A = Average coefficient of variation among replicates within laboratories
C_B = Coefficient of variation among laboratories with intra-laboratory variation removed
C_C = Coefficient of variation among PCB concentrations for a sample sent to a random laboratory with a single replicate concentration result reported

The ability to recover and measure a quantity of analyte added to a sample demonstrates the accuracy of a method. Sample C is identical in matrix to sample B, except the chlorobiphenyl congeners were added (Table 9). The data, as reported by laboratory for these two samples, is shown in the table. The values found for sample C in ppm are shown in the first line from left to right. The second line represents the data for sample B. The difference between B and C represents the amount found, and the last line represents the recovery based on the addition of 64 ppm of the 22 congeners. The data from laboratory K arrived too late to be a part of the original manuscript, but is included here.

The reproducibility of the recovery for eight of the ten labs is remarkable. The ability to quantitate levels of chlorobiphenyls, however, is very random. This implies each analyst has a high degree of discipline to measure chlorobiphenyl in matrices he's familiar with relative to his method. However, the absence of an agreed upon method or convention for the selective chlorobiphenyl detection causes widely divergent concentration to be reported.

Figure 8 gives a graphical representation of the data reported for samples A and across all labs is shown here. It appears that no single laboratory has the ability to analyze all matrices well; however, for each sample two or three of the labs generate data which is in reasonable agreement, indicating some common familiarity with the matrix and types of interferences. The data for samples C, and D are shown in Figure 9. The relative error contribution as a function of homolog (Table 10) was evaluated as follows. For sample A, the calculated mean, the lowest value, the highest value and the resulting length of the reported data by homologue is shown in the first five columns.

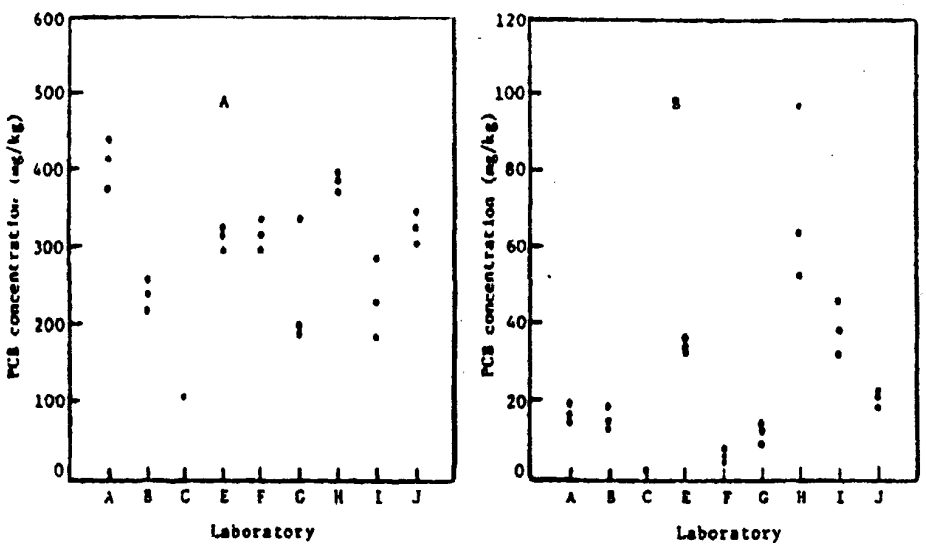
The average standard deviation, expressed in ppm, to be expected for a sample sent to a random laboratory and analyzed in a single replicate is shown in column six. The calculated relative error, expressed in percent at the 95% confidence level of each homolog's contribution to the overall sample analysis is shown in column seven. The relative error and the range as a function of homolog for each sample are shown graphically on the following figures.

For sample A, the relative error at the 95% confidence level was 68% for all homologs, 30% for the mono, and an average of 10% or the other homologs (Figure 10). For sample B, the relative error for all homologs was 184% mono was 133%, and the others averaged 28% (Figure 11). For C, 92% for all homologs, 50% for mono, and an average of 16% or the others (Figure 12). For D, 144% or all homologs, 105% for mono, and an average of 17% for the others (Figure 13). For sample E, 106% for all homologs; 65% for mono, 43% or di, and 22% or tri (Figure 14). The matrix contribution for these types of samples affects the ability to quantitate the lower chlorinated homologs, especially mono the most. Other types of matrices may affect quantitation of some of the higher homologs in the absence of suitable cleanups.

The objective of the round robin was explanatory in nature and designed to determine the degree of uncertainty associated with incidental PCB analysis. The data show that the magnitude of these uncertainties are highly significant. The impact of requesting a sample analysis for incidental PCB from a random laboratory could produce measurements far from the true value.

Table 9. Summary of Chlorobiphenyl Spike

Sample	Laboratory A	B	C	E	F	G	H	I	J	K	Mean
C	66	69	7	85	45	63	112	50	80	61	65
B	16	17	1	35	6	12	71	39	21	10	17
Amount Found -	50	52	-	50	39	51	41	11	59	51	48
% Recovery (Based on 64 ppm Added)	78%	81%	-	78%	61%	80%	64%	17%	92%	80%	75%

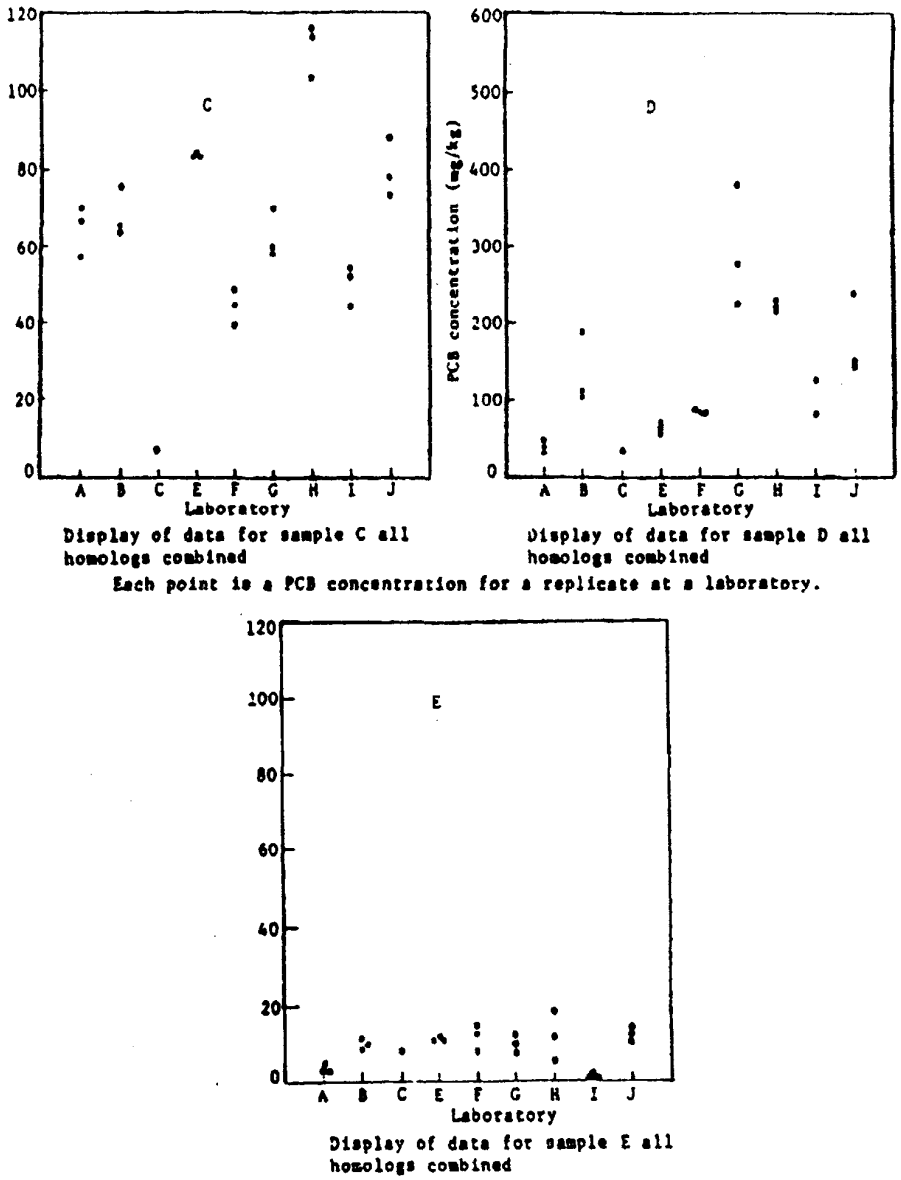


Display of data for sample A all homologs combined

Display of data for sample B all homologs combined

Each point is a PCB concentration for a replicate at a laboratory.

Figure 8. Chlorinated biphenyl concentrations as reported by laboratory for samples A and B of the Round Robin Analysis.



Display of data for sample C all homologs combined

Display of data for sample D all homologs combined

Each point is a PCB concentration for a replicate at a laboratory.

Display of data for sample E all homologs combined

Figure 9. Chlorinated biphenyl concentrations as reported by laboratory for samples C, D and E of the Round Robin Analysis.

Table 10. Summary Statistics for Sample "A" by Homolog

Homolog	Mean	Range			σ[a] (ppm)	Relative[b] Error (5)
		Low	High	Length		
1	52	18	123	105	±42	±30%
2	59	24	98	74	±22	±15%
3	60	35	96	61	±23	±16%
4	33	7	52	46	±18	±13%
5	20	0	37	37	±13	± 9%
6	16	0	31	31	±12	± 8%
7	18	0	77	77	±24	±17%
8	10	0	35	35	±10	± 7%
9	6	0	14	14	± 4	± 3%
10	11	0	21	21	± 7	± 5%

Total PCB content = 284 ppm

[a]The average standard deviation (σ), expressed in ppm, to be expected for a sample sent to a random laboratory and analyzed in a single replicate.

[b]The relative error, $\left(\frac{2\sigma \times 100}{\text{Sample Mean PCB Content}}\right)$, expressed in percent, of each homolog's contribution to the overall sample analysis.

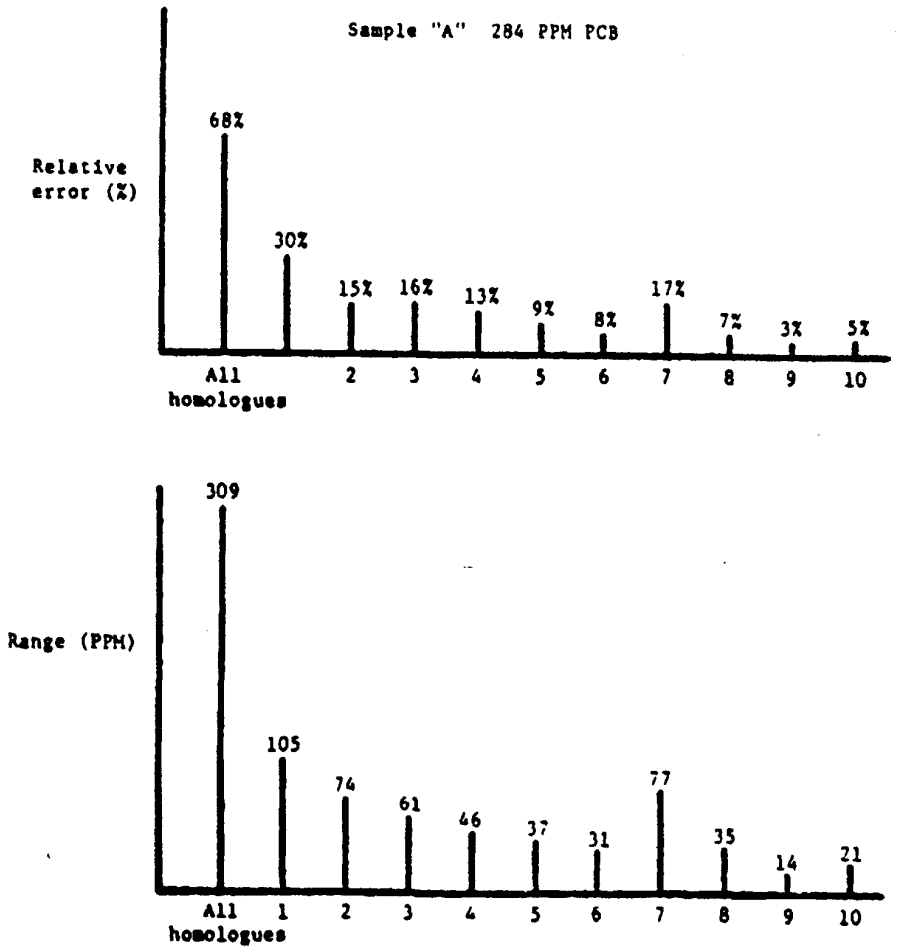
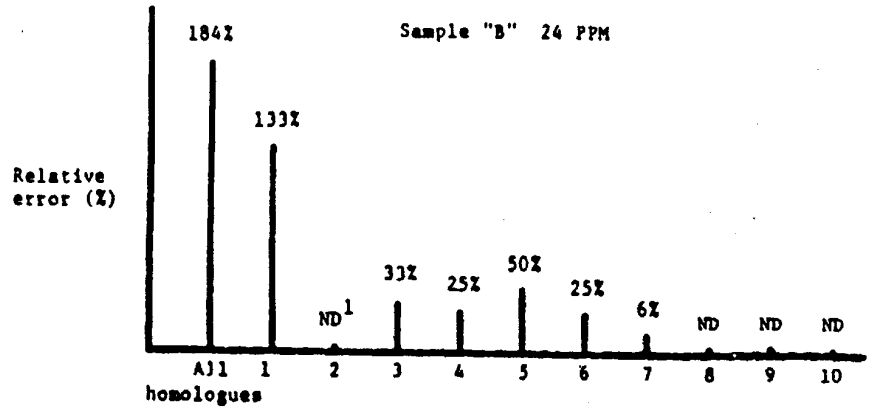


Figure 10. CMA PCB Round Robin Summary - relative error/range as a function of homologue - sample "A."



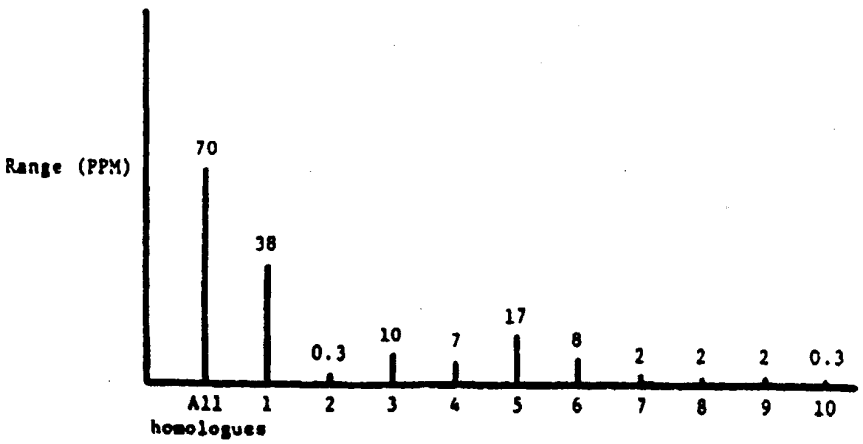


Figure 11. CMA PCB Round Robin Summary - relative error/range as a function of homologue - sample "B."

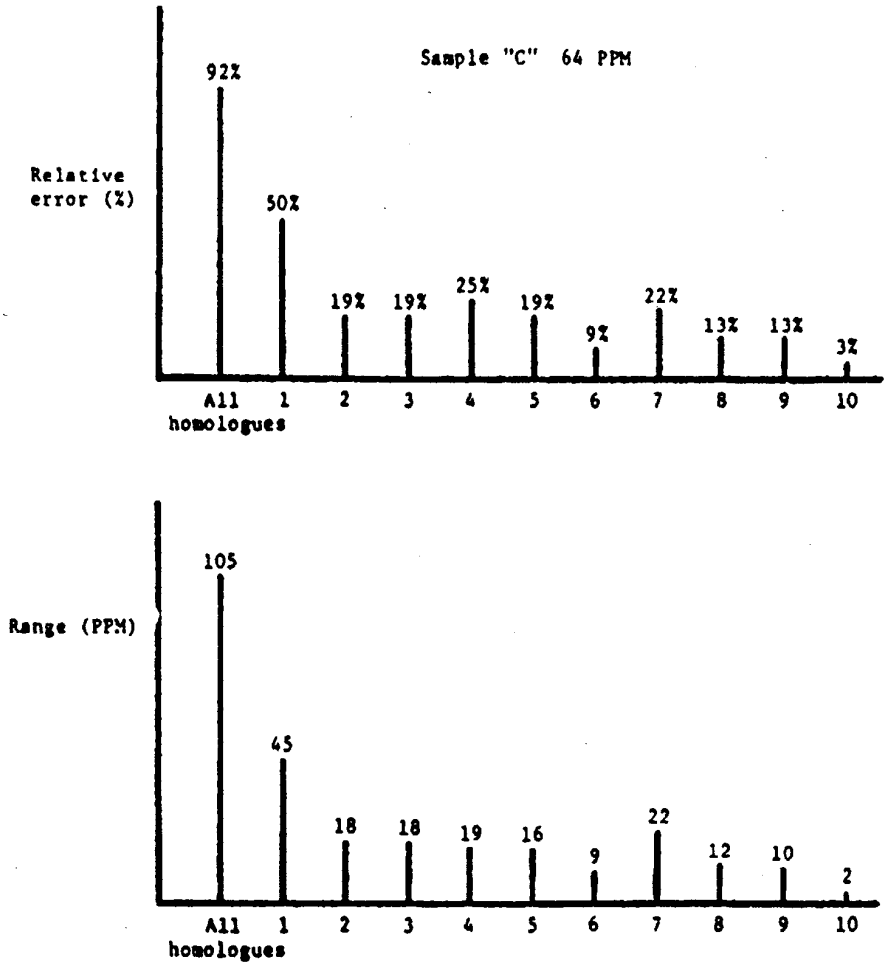


Figure 12. CMA PCB Round Robin Summary - relative error/range as a function of homologue - sample "C."

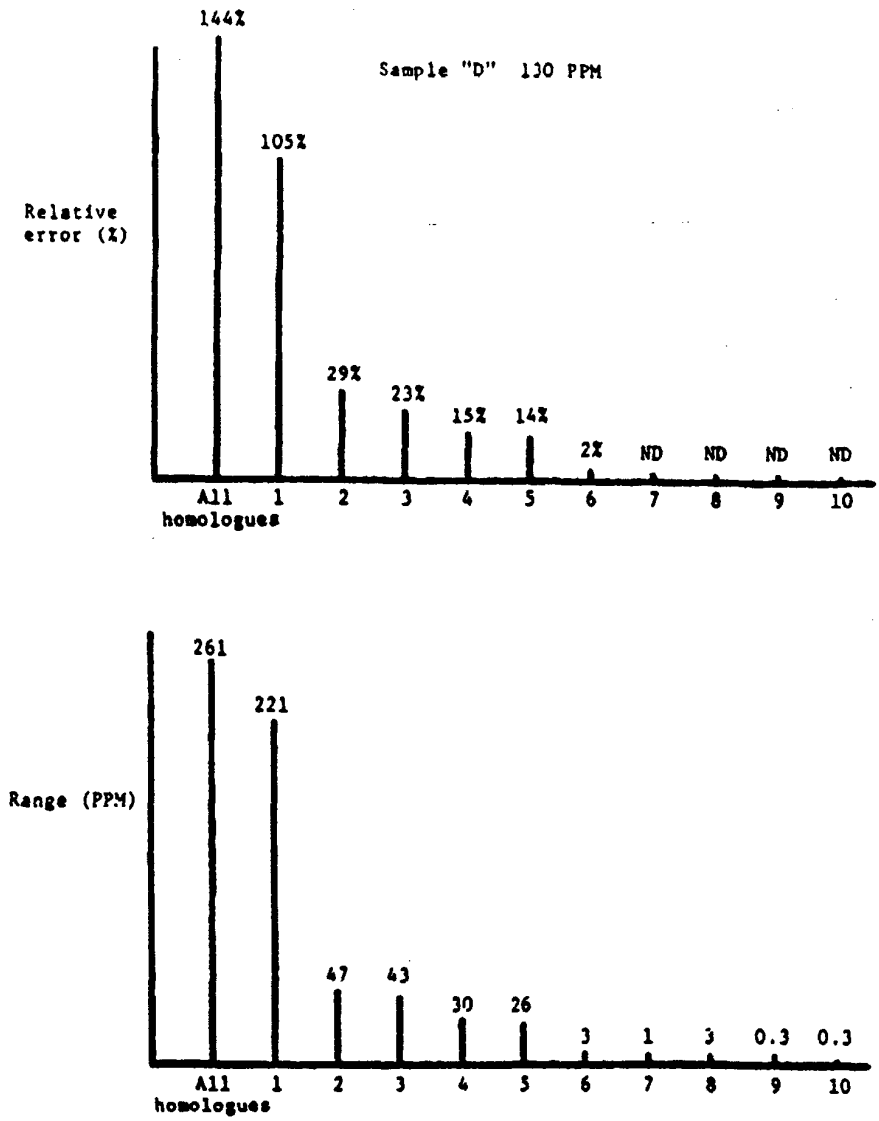


Figure 13. CMA PCB Round Robin Summary - relative error/range as a function of homologue - sample "D."

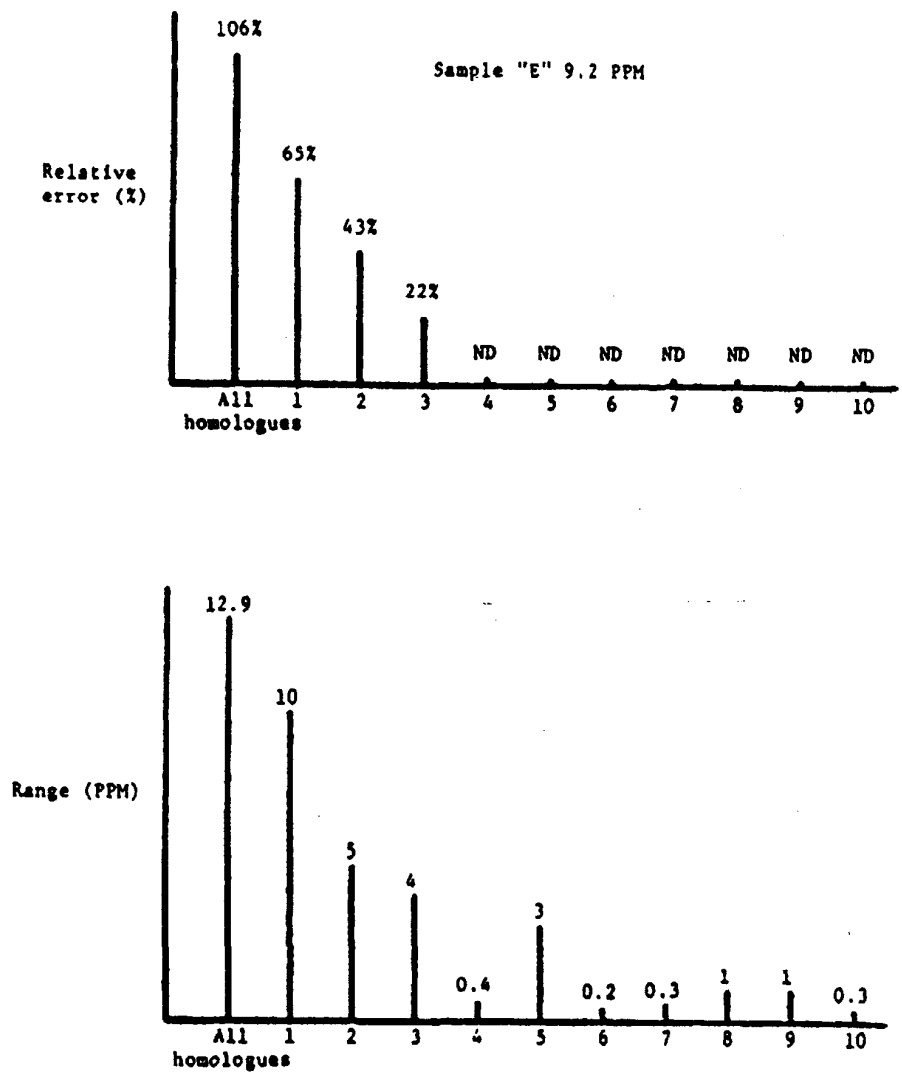


Figure 14. CMA PCB Round Robin Summary - relative error/range as a function of homologue - sample "E."

and that the use of such results for certification of a manufacturing operation, below a specified regulatory cut off (i.e., 50 ppm), could be highly misleading.

"Zero Molecules" or "The Absence of" as Defined in a Regulatory Context

From an analytical science perspective, it is important to focus on a specified regulatory level based on risk assessment. The regulatory level, in effect, defines a goal or aim point for the analytical chemist. Only then can he develop method criteria such as sample collection, preparation, cleanup, and instrumental measurement. This results in a set of orderly experiments which allows the method criteria to be obtained. The result is a validated analytical measuring device with a quantifiable degree of certainty. On the other hand, "Zero Molecules" or "The Absence of," as defined in a regulatory context are scientifically unquantifiable, and are statistically uncalculable.

McElrath and Bearman (1956) have noted the similarity in thinking between scientists and lawyers in dealing with uncertainty. Scientists express their uncertainties in terms of probabilities through statistics. The standardized degrees of certainties are 95%, 99%, or 99.9% probabilities. The lawyers use the following such phrases as their corollaries, "preponderance of evidence," "clear and convincing," and "beyond a reasonable doubt" for their degree of certainty.

The implementation of regulatory levels based on "Zero Molecules" or "Absence of" will undoubtedly introduce the following descriptions of uncertainty: "lack of evidence," "obscure and confusing," and "with considerable doubt."

EXPOSURE AND RISK POTENTIAL RELATING TO INCIDENTAL PCB

Data covering worker exposure and potential risk incidental PCB has on the environment is the subject of a recently prepared survey conducted by the Chemical Manufactures Association entitled "The Incidental Manufacture, Processing, Distribution and Use of PCB at Concentrations Below 50 PPM." (EPA 1981). The survey represents over a year of data accumulation from 85 different chemical manufacturing firms and thus, represents the current extent and fate of incidental PCB. In this survey, 135 chemical processes were described which generated a total of 13,800 lbs. of PCB per year. Ninety-five percent or 13,100 lbs. of this material is disposed of as controlled waste.

Of the 13,100 lbs. disposed of as controlled waste, 9,100 lbs. (66%) is incinerated, 1,800 lbs. (13%) is in a salt water wash discharged to a salt water reservoir surface water system, 1,600 lbs. (12%) goes to landfill, and 600 lbs. (2%) goes to ground injection.

The difference between the 13,800 lbs. of incidental PCB produced and 13,100 lbs. disposed of, represents the net pounds (700) of PCB per year contained in products manufactured by the chemical industry. Of this amount, 500 pounds (75%) are encapsulated in solid products such as paint films and polymers, or destroyed, thus inhibiting human contact and exposure to the environment. No chlorobiphenyls were reported as going into consumer products. About 490 of the 660 lbs. were reported to be in products which were listed as

"captive intermediates," intermediates, or industrial products. And finally, only 68 lbs. per year were reported entering the environment as commercial products.

In regard to workplace occupational exposure to chlorinated biphenyls, the chances are extremely low, since 99% of the poundage is generated and processed in "enclosed process systems" or "controlled release process systems," where release occurs under carefully controlled conditions.

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DISCUSSION SUMMARY

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The prepared presentation for this session was entitled Exposure Studies Relating to Industrial Processes Containing Incidental PCB. The paper addressed the incidental generation of chlorinated biphenyls in industrial processes and the measurement of the chlorinated biphenyls in process streams and products. The question of incidental generation of chlorinated biphenyls has arisen primarily in response to regulation of materials containing PCBs at greater than 50 ppm. These regulations necessitated the development of analytical techniques to identify and quantify low levels of chlorobiphenyls in a wide variety of industrial matrices.

Some of the analytical problems in the area of analysis for incidentally generated chlorobiphenyls are common to the determination of PCBs in environmental or askarel-fluid type matrices. These problems were addressed in Session 1 of this symposium. However, many of the problems are unique to the incidental generation situation. Some of the areas of particular concern are the wide variety of matrices which are encountered, the availability of appropriate standards, and the suitability of various analytical techniques for qualitative identification or confirmation and quantification in the absence of Aroclor®* fluid elution patterns, i.e., fingerprints.

SUITABILITY OF ANALYTICAL TECHNIQUES

The paper presented for this session emphasized the apparent superiority of electron impact (EI) gas chromatography/mass spectrometry (GC/MS) for the determination of incidentally generated chlorobiphenyls. Although GC/MS is more readily available than it was several years ago, electron capture gas chromatography (ECGC) is still the technique most usually identified with chlorobiphenyl analysis. The bulk of the literature which mentions chlorobiphenyl analysis has used ECGC to detect and measure commercial PCB mixtures in environmental matrices such as water, sediment, or biological tissues. There is also a body of literature which has discussed characterization of commercial mixtures using ECGC with or without other confirmatory techniques.

Most of the methodology in the literature uses the characteristic elution patterns of the various homologs and isomers of chlorobiphenyls as the primary means of qualitative identification. However, this approach is not applicable to the determination of incidentally generated chlorobiphenyls, because they are formed in processes and by mechanisms unrelated to the manufacture of the commercial mixtures. In cases of incidental generation, single isomers, isomers of a single homolog, or a variety of mixtures of isomers and homologs can be formed at trace levels. The elution pattern of these compounds obtained by ECGC cannot be recognized as chlorobiphenyls in most cases. Therefore, mass spectrometry must be used to identify the chlorobiphenyls which are present.

*Aroclor is a registered trademark of Monsanto Company, St. Louis, MO 63167.

An important question which is yet to be completely resolved is whether packed GC columns or capillary GC columns are the best for the analyses in question. (In general, the term capillary column is understood to mean fused silica capillary column.) The presentation and discussions in Session 1 emphasized the advantages of capillary gas chromatography with respect to resolution of the chlorobiphenyl isomers from one another and the separation of chlorobiphenyls from potential interferences. In fact, some analytical chemists feel that packed GC columns should be rejected completely. The ability to attain upwards of 2 parts in 10,000 reproducibility of relative retention time data with capillary columns is a strong argument for qualitative identification of chlorobiphenyls, whether as single isomers or as complex mixtures. The combination of capillary GC with mass spectrometry is certainly the most powerful technique applicable to the detection of chlorobiphenyls.

The other side of the argument is that, although the separating power and qualitative aspects of capillary GC are irrefutable, there is a paucity of validated methodology for the determination of chlorinated biphenyls using capillary columns. Since an important aspect of the incidental PCB problem is regulatory compliance, the availability and applicability of validated methodology must be considered. The accuracy and precision of capillary GC methods for PCBs have not been widely addressed in the literature, especially for cases utilizing the mass spectrometer as the detector.

In the particular case of generating analytical results for compliance purposes, the question is not so much whether to use packed or capillary GC columns, but how to generate high quality analytical results. Method validation involves all of the steps in a procedure, from sample collection through analyte measurement. There is no question that high quality data can be generated with capillary GC/MS. In some cases results of high quality also can be generated by methods utilizing packed column GC/MS. Validation results are available for many of these methods. In the final assessment, the question of data quality must be demonstrated by validated analytical methodology, and substantiated by a thorough quality assurance and quality control program, whether the method uses capillary or packed GC columns.

MATRIX CONSIDERATIONS AND CONGENER DISTRIBUTION

One of the most serious problems in the analysis for incidentally generated chlorobiphenyls is the wide variety of matrices which is encountered. In general, chlorinated solvents or any chemical systems which contain an organic compound and a source of chlorine have the potential to generate trace levels of PCBs. Certainly this statement is an oversimplification and most such processes probably do not generate PCBs, but the potential is there.

The matrix or particular process also determines the distribution of the PCBs that could be generated. For example, one might expect that only lower chlorinated congeners could be incidentally generated in a process involving a monochlorinated aromatic compound. However, in the case of phthalocyanine blue pigments, penta- and hexachlorobiphenyls are generated from the trichlorobenzene which is used as a solvent. Further chlorination of this system to produce phthalocyanine green pigment generates exclusively decachlorobiphenyl.

To a large extent, the matrix also dictates the clean-up methodology that must be used to permit accurate and precise measurement of the PCBs that are present. In the case of relatively clean solvent systems, a simple "dilute and shoot" procedure often suffices. However, in the case of the phthalocyanine pigments, an exhaustive sulfuric acid digestion of the matrix is required to free the PCBs from the matrix. (A more time consuming strong solvent extraction procedure is also available.) In this case, another problem has been documented. The sulfuric acid treatment sulfonates the lower chlorinated homologs, so that, if they had been present, they would not have been measured by the procedure.

Another problem is that the matrix in which particular incidentally generated PCBs are found can change. One manufacturer's product often is another's raw material. In such a case, the chlorobiphenyls may not be measurable by the same method used to quantitate them in the first place because the matrix may have changed dramatically. In addition, the second process may alter the distribution of congeners. This is especially a problem if one is trying to document the occurrence of PCBs in a process or to track the generation of PCBs through the process.

A similar concern arises in the processes for chemically destroying commercial PCB mixtures. As the destruction process is carried out, the mixture of chlorobiphenyls changes significantly, so that monitoring the progress or the effectiveness of the degradation scheme requires an ever-changing quantification scheme.

Finally, the matrix can affect the accuracy and precision of an analytical method in an isomer or homolog specific way. The results of the Chemical Manufacturers Association (CMA) PCB Analytical Task Group round robin discussed in the prepared paper may provide an illustration of this effect. In this particular case, the precision for the monochlorinated biphenyls was significantly worse than for the other homologs. This could be attributed to the fact that in that particular matrix, most interferences from the matrix occur in the retention window of the monochlorobiphenyls, affecting the quantification of those compounds. This effect could be expected to occur for different homologs in different matrices.

In summary, the questions involving matrix effects seem to be some of the most problematical. It seems unlikely at this point that any single analytical scheme will provide accurate and precise results for incidentally generated PCBs in all the matrices in which they might occur.

QUALITY ASSURANCE AND DATA MANAGEMENT

The importance of a comprehensive quality assurance program has already been emphasized. It is essential that only fully validated methods be used for the determination of incidentally generated chlorobiphenyls. Further, a continuous program of quality control must be used to insure that the accuracy and precision established for the method are maintained during routine use of the method.

An important concept which may have significant impact on future quality assurance programs, as well as data management, is that of pattern recognition. Pattern recognition, in the sense of visual recognition of PCB elution patterns, has been important for any years. However, the future applications of pattern recognition involve computerized systems which mathematically evaluate experimental results and cluster the results in understandable form. In the Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, Columbia, MO, pattern recognition is seen as one of the most critical and powerful means of evaluating the performance of established methodology and identifying problems when they exist.

The "patterns" which may exist for incidentally generated PCBs are not established yet. However, it is reasonable to assume that these patterns will be identified as research continues in this area. As the data base for these analyses becomes established, pattern recognition could be a valuable tool to identify patterns that might exist in various materials. The use of the samples as training sets, as opposed to using commercial PCB mixtures, for principal component analyses, shows strong potential for generating such data bases. This type of systematic approach to data management for both samples and quality control samples could help analytical chemists make sense out of what otherwise would become "a real mess."

EXTENT OF PROBLEM - EXPOSURE AND BIOAVAILABILITY

One of the issues relating to incidentally generated chlorobiphenyls is the extent of the problem. The question involves the amounts of chlorobiphenyls actually being produced, the potential exposure to these chlorobiphenyls, and the bioavailability of the chlorobiphenyls.

Although information regarding the amounts of incidentally generated PCBs is sparse, a recent survey by the CMA identified about 135 processes which could produce about 14,000 lbs. of incidental PCBs at levels less than 50 ppm. The bulk of these materials are disposed of in an EPA regulated manner. Most of the remainder is in forms not readily available for exposure, for example, the phthalocyanine pigments mentioned earlier. This is the very reason why treatments as harsh as sulfuric acid digestion are necessary to analyze for chlorobiphenyls in such matrices. Even if the CMA estimate is low by a factor of 100 or so, the amounts of PCBs produced and released by incidental generation are very low relative to the amounts still authorized for use in older electrical equipment. This point was discussed more fully by Dr. R. J. Moolenaar in Session 2: Exposure Studies - Environmental Residues and Bioaccumulation.

Other information regarding incidental generation of chlorobiphenyls has been filed with the EPA in exemption petitions. This information could also help identify specific processes or conditions which may present particular concerns. However, the information has not been published and is not readily available for review by toxicologists to evaluate the risks or potential exposure.

There are certainly cases where the environment, animals, and humans have been exposed to PCBs in nonobvious ways. Several reported examples are the ingestion of PCB-containing anti-corrosion paint by hogs, application of PCB-diluted pesticides to cattle, leakage of PCBs into a fat recycling system and

subsequent recycling into animal feed, and the related polybrominated biphenyl (PBB) incident in Michigan. However, it must be emphasized that these were gross exposures to commercial PCBs, not exposures to incidentally generated PCBs at levels less than 50 ppm.

In summary, it appears that the amounts of chlorobiphenyls generated incidentally in chemical processes is small compared to the amount authorized for use or already in the environment. However, the toxicological and epidemiological assessments of these chlorobiphenyls has not been carried out.

THE 50 ppm QUESTION

Many of the questions regarding the incidental generation of chlorinated biphenyls are concerned with the 50 ppm regulatory cut off level for PCBs. The original rules regarding the manufacture of PCBs essentially stated that PCBs manufactured at less than 50 ppm in chemical processes were not subject to regulation. The appropriateness of this cut off level has been questioned in court and the EPA is presently rewriting these regulations.

According to a former EPA employee, the 50 ppm level was established for several reasons. One concern was to establish a level that would clearly separate environmental contamination from manufacturing activities. Since sewer sludge and certain sediments showed PCB contamination at the 20 to 30 ppm level, a rule to require disposal of PCBs at a level below 50 ppm would have effected sewage treatment and dredging operations, for example. This would have created an unmanageable situation in terms of bulk of materials to be incinerated or landfilled. The EPA also felt that the Clean Water Act and Clean Air Act could effectively regulate PCBs in river sediment and sewage sludge on a case-by-case basis. Economic considerations were also involved in the decision to set the cut off at 50 ppm. Studies of costs of PCB removal showed that costs increased dramatically at about the 50 ppm level. For these reasons the regulatory cut off was established at the 50 ppm level.

There are still many questions which impact on the appropriateness of the 50 ppm cut off. The extent of the problem is certainly one concern. This was discussed in the previous section. There are also serious analytical problems which must be addressed. These were the subject of Mr. Hodges' presentation for this Session. Lastly, the toxicological and epidemiological aspects of the questions are yet to be fully explored. A great deal of research will be necessary before these questions can be resolved.

IMPLICATIONS FOR TOXICOLOGY

The increasing sophistication of analytical technology creates a serious dilemma for toxicologists. Methods utilizing capillary GC columns with electron capture, EI mass spectrometric, or negative ion chemical ionization (NICI) mass spectrometric detectors can detect subnanogram quantities of individual PCB congeners in many cases. Some of these congeners are more toxic than others. However, very few of these individual congeners have been tested for toxicity. Further, in toxicological testing most exposures are to mixtures of congeners, rather than to single congeners, and at relatively high levels compared to the ultimate detection limits of the analytical techniques.

The problem for toxicologists then becomes what to do with the analytical data, whether for commercial PCB mixtures or for incidentally generated PCB congeners or mixtures. The toxicologists need to relate dosage (or exposure level) to effect observed. From their viewpoint, the ideal situation would be to take the results of the sophisticated analytical methodology and produce some surrogate value which relates to dosage or exposure.

One possible approach to the above problem is the use of a matrix approach to format the results of analytical studies. (This approach is closely tied to structure - activity correlations discussed in Session 5 by Dr. S. Safe.) In one example, the results are reported by the number of chlorines per biphenyl ring, as well as identifying how many chlorines are ortho-substituted. By analogy to chlorinated dibenzo-p-dioxins, ortho-unsubstituted isomers have more enzyme induction activity and may be of particular concern toxicologically.

This type of approach may be a valuable interface between the analytical chemists and the toxicologists.

SUMMARY

The issues surrounding the incidental generation of chlorobiphenyls are only beginning to be addressed. Although environmental analyses for commercial PCB mixtures have advanced impressively, the analytical approaches developed for commercial mixtures or for environmental analyses should have applicability for this area. Aside from the analytical issues, there are still many questions to be answered with respect to the level and significance of exposure. In addition, the question of regulation is, as of this writing, still to be answered. It is to be hoped that an effective scientific approach to answering these questions will provide answers which will protect humans and the environment, while at the same time minimize economic burdens.

CHAPTER 5

HEALTH EFFECTS - EPIDEMIOLOGY

This paper summarizes the results of seven cross-sectional epidemiologic studies dealing with health effects of PCBs, and three cohort mortality studies which contained data on the carcinogenicity of PCBs in humans. The findings from the cross-sectional studies are analyzed according to the following categories: dermatologic effects, liver function, fat metabolism, other objective findings, and reported symptoms and illnesses. The reported cohort mortality studies result in terms of numbers of death attributed to various types of cancer. The paper concludes with a general discussion on the strengths and weaknesses of each study, and the cross-study results which can be drawn forth.

During the first part of the discussion summary, a paper is presented reviewing possible human reproductive effects of PCBs. The data includes results obtained from the Yusho incident in Japan, and studies of several groups of individuals previously reported on by William Gaffey. The discussion addresses the measured variables of days of gestation prior to birth and birth weight. A second study examining the relationship of PCBs to hypertension is presented.

RECENT EPIDEMIOLOGIC STUDIES OF PCBs

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Before the Yusho incident in 1968, published studies of the health effects of PCBs, with the exception of Meigs et al. (1954), were essentially clinical studies of occasional accidental severe exposures. In the following decade ten epidemiologic studies were published (in addition to the many reports on the Yusho incident itself), all but one of which dealt with occupationally exposed populations, and only one of which studied the relationship between cancer and PCB exposure (Gaffey 1982).

Since 1978 there have been ten epidemiologic studies of the health effects of PCBs. Seven of them are cross-sectional investigations of symptoms and biochemical parameters. Of these, five are occupational, one is a study of both occupational and nonoccupational exposure, and one is nonoccupational. The remaining three reports are cohort mortality studies of occupationally exposed groups. In addition, there has been a report on cancer mortality in the Yusho population.

This paper briefly summarizes the status of the epidemiologic evidence on health effects as of 1978, and reviews and evaluates the subsequent studies in detail.

THE EVIDENCE AS OF 1978

The Yusho incident of 1968 at first appeared to be a classic example of the effects of a massive ingestion of PCBs. Over 1000 Japanese became ill after eating a cooking oil contaminated with Kanechlor 400, a PCB compound of Japanese manufacture. The most common acute symptoms observed were hyperpigmentation and acne-like lesions, central nervous system symptoms, discharge from the eyes, and vomiting and diarrhea. These symptoms were dose related, and some of them persisted for as long as six years. Laboratory tests showed disturbances of liver function and fat metabolism, and there were clinical reports suggestive of some abnormalities in the children of Yusho mothers (NIOSH, 1977).

As of the end of 1977, 51 deaths among Yusho patients had been identified, with an apparent excess of cancer deaths (Urabe et al. 1979). However, the data were not adjusted for age, and the completeness of ascertainment of the deaths is not known. The elapsed time from the original incident to the reported deaths (one decade) was short enough to cast doubt on whether the cancers could in fact have arisen from the Yusho exposure.

In any case, in the decade following the Yusho incident analytic techniques for identifying PCBs and allied compounds improved considerably, and the cooking oil was reanalyzed. The new analyses showed that the Yusho patients had in fact ingested about the same amount of polychlorinated dibenzofurans (PCDFs) as of PCBs, in addition to polychlorinated quater-phenyls

(PCQs). Current determinations of blood and tissue levels of these other compounds in Yusho patients have shown total levels about equal to that of PCBs (Kimbrough 1980). It therefore appears that the epidemiologic findings in the Yusho incident should properly be attributed to the more toxic PCDF rather than to PCBs.

There were nine other published cross-sectional studies of symptoms and biochemical parameters related to PCB exposure, one of which was nonoccupational, and one occupational study of cancer morbidity and mortality.

In summary, the occupational studies found chloracne or other dermatitis, and mild liver function abnormalities in the absence of clinical illness, to be associated with PCB exposure. Two studies of cholesterol levels gave contradictory results, and one study of triglyceride levels showed an increase. The nonoccupational study showed no association between blood PCB levels and any of the Yusho symptoms, but did not examine liver function or fat metabolism.

The cancer study found an apparent excess of malignant melanoma based on three cases, but was later withdrawn because of concern about whether the exposed population had been correctly identified.

RECENT STUDIES

The seven cross-sectional studies completed since 1978 address a broader range of health effects than did the pre-1978 studies, and in some cases used formal statistical techniques to take account of confounding variables such as age, sex and weight. Two of the studies, Fischbein et al. (1979) and Smith et al. (1982) evaluated their findings in relation to higher chlorinated and lower chlorinated PCBs separately. The studies' findings can be classified under five general headings: dermatologic effects, liver function, fat metabolism, other objective findings, and reported symptoms and illnesses. The cohort mortality studies, although they collected data on all causes of death, were concerned primarily with cancer.

Dermatologic Effects

Table 1 summarizes the results of the five studies that reported on dermatologic effects. The negative study of Baker et al. (1980) examined 18 exposed workers, 19 members of their families, and 89 community residents with exposure to fertilizer containing PCBs. The rest were occupational studies, two of which found chloracne and all of which found dermatitis.

Fischbein et al. (1979) reported that 50 percent of 326 capacitor manufacturing workers reported a history of dermatological symptoms, the most common being a rash. Those with symptoms had higher blood levels of high chlorinated PCBs. Maroni et al. (1981 II) reported ten cases of dermatitis (five diagnosed as active or past chloracne) in 80 exposed workers, but provided no further data on the blood PCB levels of those with dermatitis versus those without. Chase et al. (1982) found increased dermatitis, and some chloracne, in the most exposed group out of 120 railroad maintenance workers, but within the group found no significant association with blood or fat PCB levels. Smith et al. (1982), in a study of 92 employees of two

Table 1. Studies of Chloracne and Other Dermatitis in Relation to Blood PCB Levels*

Study	Chloracne	Other Dermatitis	Dose Related	Adjusted for Other variables
Fischbein et al. (1979)	N	Y	Y	N
Baker et al. (1980)	N	N		N
Maroni et al. (1981 II)	Y	Y	?	N
Chese et al. (1982)	Y	Y	?	N
Smith et al. (1982)	N	Y	N	N

* N = Not found
Y = Found
No entry = Not reported
? = Equivocal finding

utility companies, found no consistent association between dermatitis and either high or low chlorinated blood PCB levels.

Although the data are not consistent, they suggest that there may be a gross dose response relationship between dermatitis and blood levels of PCBs, possibly complicated by variations in individual susceptibility and in work habits that may affect absorption of PCBs through the skin.

Liver Function

Table 2 shows the results of the six studies that investigated liver function. The two nonoccupational studies showed no liver function anomalies associated with blood PCB levels. The first one did not adjust for confounding variables (Baker et al. 1980). The second, a study of 458 residents of a community with high environmental levels of PCBs, found an association which disappeared when age and alcohol consumption were taken into account (Kreiss et al. 1981).

Of the four occupational studies, three showed various liver function anomalies related to blood PCB levels. Chase et al. (1982) adjusted the results for age. Smith et al. (1982) adjusted the data for age and sex. The latter study showed a significant association of liver function anomalies with low chlorinated blood PCBs in one of the two plants studied but not in the other. Maroni et al. (1981 II) found liver abnormalities associated with elevated blood PCB levels, but the liver abnormalities were defined as liver function anomalies or liver related symptoms or clinical findings of abnormality. They state that "only in a few cases was a well-defined liver failure (presence of symptoms, hepatomegaly, and abnormal liver findings) present."

Fischbein et al. (1979) found no liver function anomalies associated with exposure, and in fact commented on the "paucity of abnormal results" in their biochemical studies.

With the exception of Maroni et al. 1981, the occupational studies agree in finding few or no liver function abnormalities, and no associated clinical illness.

Fat Metabolism

Table 3 summarizes the results of the five studies that examined fat metabolism. Fischbein et al. (1979) found no association between cholesterol or triglycerides and blood PCBs. Kreiss et al. (1981) found an increase in cholesterol with increasing blood PCB levels, but no relationship of triglycerides to blood PCBs when an adjustment was made for cholesterol level. Two other studies, Baker et al. (1980) and Chase et al. (1982) agree that cholesterol is not associated with blood PCBs but that triglycerides are.

Smith et al. (1982) found cholesterol levels to be positively associated with low chlorinated PCBs in one of the two plants studied. Triglycerides were positively associated with high chlorinated PCBs in one plant and negatively associated in the other, where they were positively associated with low chlorinated PCBs.

Table 2. Studies of Liver Function in Relation to Blood PCB Levels*

Study	Abnormalities	Dose Related	Adjusted for Covariables
Fischbein et al. (1979)	N		N
Baker et al. (1980)	N		N
Kreiss et al. (1981)	N		Y
Maroni et al. (1981 II)	Y	Y	N
Chase et al. (1982)	Y	Y	Y
Smith et al. (1982)	Y	Y	Y

* N = Not found
Y = Found
No entry = Not reported

Table 3. Studies of Fat Metabolism in Relation to Blood PCB Levels

Study	Total Cholesterol	Triglycerides	Adjusted for Covariables
Fischbein et al. (1979)	N	N	N
Baker et al. (1980)	N	Y	N
Kreiss et al. (1981)	Y	N	Y
Chase et al. (1982)	N	Y	Y
Smith et al. (1982)	N	N	Y

* N = Not found
  Y = Found

The preponderance of evidence is that cholesterol is not associated with blood PCBs. There is no obvious explanation for the association of cholesterol and blood PCBs found by Kreiss et al. (1981). However, their use of cholesterol as an adjustment factor for triglycerides implies that they view cholesterol as an independent variable that predicts triglycerides. The results of Baker et al. (1980) and Chase et al. (1982) do not support this view, since they show that the putative predictor, cholesterol, is not associated with blood PCB levels while triglyceride levels are. The association between triglycerides and blood PCB levels appears to be ambivalent at the least.

Other Objective Findings

Fischbein et al. (1979), Baker et al. (1980) and Maroni et al. (1981 II) examined blood chemistry and found no association with blood PCB levels.

Kreiss et al. (1981) found a statistically significant positive association between diastolic blood pressure and blood PCBs after adjusting for cholesterol, triglycerides, smoking and race. However, Smith et al. (1982) reported no such association.

Warshaw et al. (1979) reported decreased vital capacity in 243 capacitor manufacturing workers compared with published reference standards. However, most of the study population were current or former smokers, while the reference standard is based on a non-smoking population. The effects of smoking could be sufficient to explain the findings.

Reported Symptoms and Illnesses

Six of the studies investigated a range of symptoms and illnesses. Two of them reported positive findings in occupationally exposed populations. Fischbein et al. (1979) reported a history of gastrointestinal symptoms in 18 percent of 326 capacitor manufacturing workers, a prevalence of from 3.0 to 15.2 percent of various musculoskeletal symptoms, and a prevalence of from 4.8 to 27.8 of various neurological symptoms. These were, however, unrelated to blood PCB level or duration of employment. Maroni et al. (1981 II) reported eight cases of gastrointestinal complaints in 80 exposed workers with no indication of whether there was a relationship to blood PCB level or duration of employment. They also reported two bleeding haemangiomas and one case of chronic myelocytic leukemia.

The other four studies reported no PCB related findings. Specifically Baker et al. (1980) found no relationship with any of the following; fever, weight loss, anorexia, fatigue, headache, eye irritation, cough, shortness of breath, nausea, vomiting, diarrhea, abdominal pain, arthralgia and persistent rash. Kreiss et al. (1981) reported the same thing for prevalence of illness or weight loss in the previous year, use of medication, use of medical care, history of heart disease, and percentage of pregnancies ending in miscarriage, stillbirth or infant death.

Chase et al. (1982) failed to find any evidence of organ toxicity in a review of the medical histories and physical findings in a group of exposed railroad maintenance workers, and Smith et al. (1982) reported no consistent

dose-dependent increase in either symptoms or past illnesses. Their inquiry covered digestive, respiratory, central and peripheral nervous system, dermatologic, and emotional symptoms.

The evidence shows no PCB related symptoms or illnesses, since the two reports out of six that mentioned symptoms failed to show an association with level or duration of exposure.

Carcinogenicity

Table 4 shows the major findings of cancer mortality in three cohort studies of PCB exposed workers. Brown et al. (1981) studied a cohort of capacitor manufacturing workers in two plants who had been exposed at least three months between 1946 and 1975 inclusive in one plant or between 1940 and 1975 inclusive in the other. Follow-up was more than 97 percent complete as of the end of 1975, and expected mortality was calculated from U.S. population rates. Nonstatistically significant excesses were found for rectal cancer, based on four deaths, and liver cancer, based on three deaths. The rectal cancers showed a slight increase with an increase in the latency period, but the liver cancers showed no consistent trend. There were no increases in mortality from these causes associated with increasing lengths of exposure.

Bertazzi et al. (1981) also studied a cohort of capacitor manufacturing workers in a plant near Milan. The cohort consisted of every person who had worked for at least six months between 1946 and 1970, inclusive, except for clerical workers. Follow-up was over 98 percent complete as of the end of 1978, and expected mortality was based on rates in the city where the plant was located. Data were analyzed separately for male (290) and female (1020) workers. A statistically significant excess mortality from all cancers, based on eight deaths, was found in male workers, and a statistically significant excess for all causes was found in female workers, based on 15 deaths. There were nonsignificant excesses of lymphatic and hematopoietic cancer in both sexes, based on four deaths, and of digestive cancer in males, based on three deaths. The sites involved in the digestive cancer deaths were stomach, pancreas and biliary tract. No analysis by duration or latency of exposure was attempted because of the small numbers involved. The authors note that the cohort was very young, and that their follow-up will be continued.

Zack et al. studied all hourly male workers who had been employed in the manufacture of PCBs in a general chemical plant for at least six months between 1945 and 1965 inclusive. Follow-up was approximately 99 percent complete (one person was lost) as of the end of 1977, and expected mortality was calculated from U.S. population rates. There were eight cancer deaths. The only noteworthy finding was a non-statistically significant excess in lung cancer based on four deaths. No liver cancer deaths were found.

DISCUSSION

Doll (1981) has suggested criteria for establishing carcinogenicity from epidemiologic evidence. They are similar to those proposed by the International Agency for Research on Cancer (1980) and are essentially as follows: (1) there is an excess in exposed groups beyond what can be expected by chance, (2) there is an appropriate relationship with dose or duration of

Table 4. Inconsistencies in Mortality Studies of Cancer in PCB Exposed Populations

Study	No. Studied	No. of Deaths	Principal Findings
Brown et al. (1981)	2567	163	Liver Rectum
Bertazzi et al. (1981)	1310	27	Digestive Lymphatic and hemo-topoietic
Zack et al. (in preparation)	89	30	Lung

exposure, (3) there are no known biasing or confounding factors, (4) the association is observed repeatedly in different circumstances. The development of these criteria was stimulated by concern to provide generally accepted guidelines for establishing carcinogenicity, but they apply equally well to other health outcomes.

Criteria for establishing the absence of a health effect are inherently more difficult. The cliche that it is impossible to prove a negative is true but misleading, in the sense that it is also impossible to prove a positive association by means of epidemiologic studies. A statistically significant positive finding allows us to conclude that an association exists, with known probability that the conclusion is wrong. Repeated positive studies reduce that probability in a manner that can be calculated, but there remains a chance that the finding is false. On the other hand, a negative study allows us to conclude that there is no association, with a probability of error that depends on how great an association really might exist. Repeated negative studies reduce this probability, but there is always a large probability that a very small effect might not be detected. Therefore both positive and negative studies carry a risk of error, although in both cases the strength of the evidence may be such as to make that chance negligibly small.

Cross-sectional Studies

There is a reasonable consensus that dermatitis is associated with occupational exposure to PCBs, although the dose response relationship has not been established in all of the studies. Two nonoccupational studies agree that there is no dermatitis associated with environmental, i.e., nonoccupational exposure. The difference in the results of the occupational and nonoccupational studies is consistent with the order-of-magnitude differences in the exposures of the two groups.

There is a similar preponderance of evidence that mild liver function anomalies are associated with occupational but not with nonoccupational exposure. One occupational study found associated clinical symptoms, but the remaining three studies found no detectable clinical illness. By Doil's criteria of consistency, it is very unlikely that clinical illness is associated with the anomalies found in occupationally exposed populations.

Findings concerning cholesterol levels again show a preponderance of evidence that cholesterol levels are not associated with blood PCB levels, even though one nonoccupational study did report an association.

The situation with respect to triglyceride levels is slightly more ambiguous. Two out of five studies, including one nonoccupational study, found a relationship with blood PCBs, so that the possibility that such a relationship really exists must be considered.

Studies of blood chemistry were uniformly negative. Two studies of diastolic blood pressure gave contradictory results. One study of pulmonary function suffered from a failure to account for smoking so that its findings cannot be attributed to PCB exposure. None of these areas of study appear to show any relationship to PCB exposure.

None of the six studies of reported illnesses and symptoms constitute evidence by Doll's criteria. Four studies showed no relationship of a wide range of symptoms to PCB exposure. Two occupational studies reported various symptoms, but did not show that they were related to blood PCB level or duration of employment.

Cohort Cancer Studies

The most noteworthy characteristic of the three mortality studies is that they do not agree with each other. Excess cancer of the liver, rectum, stomach, pancreas, biliary tract and lung are each found in one of the studies but in none of the others.

Although the power of the studies is limited (the most powerful one, Brown et al. (1981) has a probability of about 0.33 of detecting a threefold excess in liver cancer mortality), the lack of a latency relationship in that study and the complete absence of liver cancer in the other two studies strengthens this power in a way that cannot be quantified precisely.

The average duration of follow-up in each of the three studies was 15.3 years Brown et al. (1981), 15.7 years (Maroni et al. 1981) and 20.2 years (Zack et al.). Further follow-up, especially of the first two studies, would provide the longer latency and greater number of deaths that would increase the power of the studies. Nevertheless, the data available at the present time do not provide the consistent, exposure related results that would justify concluding that PCBs are carcinogenic in human beings.

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DISCUSSION SUMMARY

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Following the discourse of Dr. Gaffey, Dr. Philip Taylor presented preliminary results of a study he conducted in conjunction with investigators from the New York State Department of Health for the National Institute of Occupational Safety and Health. In this study, the effects of PCBs on reproduction in occupationally exposed women were investigated. This study was undertaken because in the Yusho episode (accidental ingestion of rice oil contaminated with PCBs, chlorinated dibenzofurans and quarterphenyls) 11 women were pregnant at the time of exposure, two of these women had miscarriages and nine had live births. Three of the infants were light for age and eight of the nine had various symptoms of fetotoxicity, such as eye discharge, cola-colored skin, and erupted teeth. In addition, fetotoxicity has been produced in a variety of animal species.

The cohort of women that was studied were workers for the General Electric Company at two facilities in Upstate New York south of Lake George. The two facilities were located in adjacent communities and have been involved in the manufacture of capacitors since 1946. There have been roughly 7400 individuals employed in those communities between 1945 and 1975, of whom about half were females. A cohort of women was studied whose personnel records indicated they had taken leave because of pregnancy.

Between 1958 and 1975, there were 388 pregnancies in 354 women that resulted in live births within the State of New York, and these became the object of the investigation. The information on birth weight, maternal age, parity, year of birth, race, sex, and date of last menstrual period was obtained from birth certificates, and where necessary hospital records and physician records were reviewed.

From a file of all single live births in the surrounding two-county area, a single control was selected for each of these births occurring to a capacitor worker matched on maternal age and parity precisely; year of birth plus or minus a couple of years; and race. The controls were cross-checked against the personnel files of the GE records to assure that they had never worked at GE. The cohort was limited to persons who had worked at least three months for GE and were divided into high and low exposure groups.

High exposure areas were those parts of the plant in which there was direct contact with PCBs during or after the introduction of PCBs into the capacitor. The areas of the plant with low exposure consisted of some women with clerical jobs which presumably would have no contact with the PCBs and women from the manufacturing process prior to the introduction of PCBs. In 1975, area samples taken in one survey showed that the values in the high areas, following the impregnation of PCBs were in the neighborhood of 679 $\mu g/m^3$ and in the lower areas they were 260. In 1977, the areas of high

exposure had an average value of 310 and the areas of low exposure of 227 $\mu g/m^3$.

Personal air samples were only done during a NIOSH industrial hygiene survey in the high areas and averaged 168 $\mu g/m^3$ air. Air samples taken immediately outside the plant averaged in the neighborhood of 6 $\mu g/m^3$ while ambient air levels are 0.1 $\mu g/m^3$ or less.

When the birth weights of infants from the low exposed group were compared to county controls, it was determined that the 337 infants of mothers in the low exposure group had birth weights that were 66 grams higher than the birth weights of the matched county controls. A comparison of gestational ages showed that the 51 neonates from high exposed women had a mean decrease of 3.4 days in gestational age and the mean difference of their birth weights was minus 95 grams when compared to county controls. This difference was not statistically significant.

However, when 51 neonates of high and 337 neonates of low exposed females within the plant were compared, the neonates of the high exposed group weighed an average of 153 grams less than the neonates of the low exposed group. This difference was statistically significant. When the data was adjusted for gestational age, it was found that the difference in weight was largely due to differences in gestational age. Overall, 13.7% of births occurred before 37 weeks of gestation in the high exposure groups and 5.6% in the low exposure group. The point estimate for the rate ratio was 2.4 with 90% confidence intervals that did not cover one. The meaning of these results, a reduction in gestational age of approximately one week and a reduction in birth weight of about 153 grams, when high and low exposure groups were compared within the plant is open to interpretation in terms of biological implications. This study was the only attempt to investigate reproductive outcomes in PCB exposed females.

All studies discussed by Dr. Gaffey in the presentation that preceded Dr. Taylor's were either cross-sectional studies in predominantly adult populations or mortality studies. Dr. Gaffey took the liberty of comparing studies that were basically quite different. Two of them were general population-type studies; the one by Baker, et al. of CDC, was done in Bloomington, Indiana. A general population that had been exposed to PCBs in soil was compared to a control population. This population had no higher blood levels of PCBs than the comparison group. It is therefore not surprising that no striking health effects could be associated with PCB exposure. The only exceptions were a small group of 18 workers or former workers who had had somewhat higher exposures.

Another study Dr. Gaffey mentioned was also a CDC study of a predominantly black population of the small town, Triana, Alabama. This population had had exceptional exposure to DDT residues, but in addition, when serum samples were analyzed, PCBs were also found in high concentrations in some of the people. A total of 458 people were examined, and the range of the PCBs in that group in serum was 3.2 to 157.9 $\mu g/l$. There were 98 persons in this group that had levels higher than 30 $\mu g/l$. There was no extensive PCB contamination in the town of Triana. The PCB blood levels in these people could be associated with the consumption of fish. As far as we could determine, the

PCB concentrations in fish had usually not exceeded FDA guidelines for the past ten years before the study was done. An additional important finding in this study was that as the age of the people increased, their PCB blood levels seemed to increase, and in females the PCB blood levels were lower than in males. According to a recent paper by Mary Wolff, there is also an increase in PCBs in adipose tissue with age. Dr. Chase in a recent publication, which was one of the papers reviewed by Dr. Gaffey, found that the workers with longer exposure had higher PCB body burdens.

The incidence of hypertension in this black population from Triana studied by Kreiss et al. was 30%. At a mean age of 24 years, this is a higher incidence than what has been reported among blacks. After adjusting for other confounding variables for hypertension, such as weight, cigarette smoking, age, alcohol consumption, there was still a small contribution which had to be attributed to PCB exposure. More studies need to be done to determine whether these findings can be verified. This population is quite different from the population that Dr. Blair Smith examined where no association with high blood pressure was found. In that population, the mean age was 40. Exposure was occupational in nature and most of the workers were white.

In addition, Dr. Gaffey discussed three mortality studies. All of them had in common that the number of available death certificates was very small. Even though small excesses of cancer were found, this information has to be taken with caution because of the limitations of the studies. Mortality needs to be examined further to determine what the significance of these findings is. If the latency period for the induction of cancer in humans is 20 to 40 years, a sufficient amount of time has not elapsed to determine whether exposure to PCBs is a risk factor in the development of cancer.

Although PCBs were first manufactured in the early part of this century, we are now beginning to see a larger population that has been exposed to PCBs for 10, 15, and 20 years or longer. This population will have to be followed further before any decision on the incidence of cancer in humans can be made.

Following these general comments, many different points were raised by the attendees.

Dr. Taylor was asked whether the children whose birth weight he reviewed were followed after birth. However, since his study was a record search study, this was not possible nor was it possible to control for confounding variables. It was, however, possible to establish that 11 children had died up to the age of 5. Seven of those deaths occurred among the 388 county controls and 4 occurred among the infants of the 377 low exposure group of the GE workers. In addition, it was again pointed out that the low exposed group and the high exposed group in the GE plant were not matched but adjustments for variables were made in the analysis of the data.

The point was made by Dr. Mary Wolff that Dr. Gaffey's statement about smoking among the workers of the Warsaw studies was irrelevant since the findings of restrictive pulmonary function tests made in that study were usually not associated with smoking. However, epoxies were used in that plant. This may be an important confounding variable.

Additional attempts to quantify exposure in the two GE plants studied by Dr. Taylor were made by Mary Wolff. The lower chlorinated biphenyls in people were strongly correlated with air levels while the higher chlorinated biphenyls were not.

Dr. Gaffey pointed out that the duration of followup in two mortality studies reviewed in his presentation (Brown and Bertazzi) was about 15-1/2 years and in the Zack study about 20 years.

So, the result is that the mortality rate, for example, in the Brown study for males was about 5.3 per 1000; in the Bertazzi study about 2.79; but among males Brown's rate was 3.8; Bertazzi's rate was 0.48. It seems to be an extraordinarily low mortality rate and yet it was almost 100% more than expected. It is very difficult to explain.

Zack's rate for males was 12.6 per 1000 which is understandable because the cutoff date for admission to the study was 12 years before the end of it, but it is puzzling. In the Bertazzi data the cohort is admittedly young. His males have a lower rate than Brown's, about half the rate, but his females have about 1/8 the rate. It is very puzzling to him.

Since in the Triana study (Kreiss, et al, 1981) exposure to PCBs and DDT residues had occurred, the question was raised whether the effects of DDT residues and PCBs could be additive, and it was pointed out that the analysis of the data did not seem to indicate this.

Dr. Blair Smith, National Institute for Occupational Safety and Health, indicated that in capacitor plant workers which he had studied the mean serum PCB levels of the lower chlorinated homologues were on the order of 500 µg/l with a background level of 10 to 15 µg/l. Yet, the mortality data from the Brown study of a plant which was similar were equivocal. He is therefore left in a quandry about trying to impute some human toxicity to PCBs in the occupational exposure situation.

In that regard Mr. David Brown asked him to say that he was planning to follow up on that cohort which has been followed through 1975. He will be following it through 1980. With additional years of followup, the study will statistically become more powerful and possibly some of the borderline excess risks for cancer may turn out to be statistically significant or possibly not. Dr. Smith went on to point out that in those studies where the magnitude of the association between PCB serum levels and biochemical tests was reported in a quantifiable way that the amount of variation was in general no more than about 10%. In regard to liver function tests and serum lipid or plasma lipid abnormalities, the associations appear weak.

When Dr. Smith analyzed his data obtained from capacitor workers blood pressure, unadjusted for age, was correlated with serum PCB level; but when it was age adjusted, the correlation went away. At present, it is not possible to explain the discrepancy between his study and that by Kreiss, et al. (1980).

Dr. Gaffey then made the point that the Brown and Jones' study has some data for longer latency periods, and the cancer data suggests no trend. He gave vinyl chloride as an example where, with these kinds of studies, some-

thing would have been seen. It was noted by Dr. Kimbrough that in the Brown and Jones study there seemed to be a trend for rectal cancer, and since vinyl chloride causes such an unusual tumor, it is much more easily detected. In addition, she pointed out that basically analyzing death certificates is a very insensitive tool.

Whereupon Dr. Taylor objected, indicating that relative to the other end points presented here, they are as sensitive. He also indicated that continuing as a former worker from CDC located in the New York State Health Department, he would be looking at and expected to have available information within the next year on the entire cohort of 7400 and some odd workers of the GE cohort. At this point, 750 deaths have been identified which will make the issue of arguing about thirties and forties reasonably moot for the particular PCB compounds—that exposure was incurred at those particular facilities.

Dr. Gaffey in further commenting on the Brown study pointed out that there were only three liver cancer deaths; it is fairly difficult to show a trend with only three observations. The Brown study was, in terms of statistical power, a relatively weak study. It will become more powerful with increasing length of followup. As epidemiologists, we are dealing with highly variable outcomes fraught with error, both due to inherent biological variability and variability due to analytical technique and mediocrity of the recording of information on death certificates. Given the inherent dirtiness of the data, how refined does our measurement technique have to be in terms of separating out specific PCB isomers and homologues? In place of a single number, the proliferation of additional numbers as predictors is confusing.

Whereupon Dr. Taylor stressed that whenever randomly sloppy data are analyzed, the bias is always in the null direction. If a difference is demonstrated with sloppy data, it is more likely that it is true than it isn't. However, if a difference is not demonstrated and the data are sloppy, then it must be determined whether the inability to establish a difference is related to poor measurement techniques. Mr. Bob Noonan with Amtrak asked Dr. Gaffey whether, in trying to explain the apparent contradictions and inconsistencies in findings in the cross-sectional studies, was an attempt made to compare PCB dosage levels and mode of exposure. Dr. Gaffey stated that he did not attempt to do that.

Dr. Gaffey, when asked by Dr. Chase whether he felt that the studies he reviewed were not adequate stated that the cross-sectional studies were quite adequate and that the mortality studies are more adequate than has been suggested; and that the problems that have been raised about the power of the tests are beside the point because a complex of things is evaluated. The power is examined to detect excess mortality or a trend. In the studies that do not show an excess, it is determined whether there is something that is almost there, but there appears to be nothing of this kind. Maybe as these studies are followed up further, the evidence will become even stronger, but the evidence does not indicate a carcinogenicity problem with PCBs or other problems beyond the dermatitis and the marginal liver abnormalities.

According to Dr. Chase, when dealing with the issue of cancer with a long latency period exceeding the latency periods allowed for in any of the three cross-sectional studies, the studies were not sufficiently powerful, either

collectively or individually, to detect an increased risk. Further, there may not be a trend in the liver cancers, but there is an excess of liver cancers in the Brown study. The excess is conceded by Brown as not being statistically significant. It is of interest that there is an excess of liver cancer in the Yusho population as well, again, not statistically significant, and the conclusion is that a sufficiently powerful study is not yet available to say anything definite. This has been said by a couple of other speakers.

Dr. Gaffey responded that he did not mention the mortality in the Yusho population because he discounted it as a PCB-related phenomenon. It is not known out of what population it arose because as time has gone on, the definition of Yusho has changed, and the denominator has changed in ways that are not predictable. Secondly, the mortality data from the Yusho study have not been age adjusted. Thus it is not possible to determine what percentage is too high since comparisons with an age-adjusted population cannot be made. In other words, this is not an age and time-adjusted proportional mortality study; and third, the elapsed time interval, at least from the Yusho incident until the first mortality figures are given was ten years. For the Yusho deaths, the question is whether a latency period of less than ten years, may have been too short to cause the cancer. As far as Brown's study is concerned, even if his excess in liver cancer were statistically significant, it should not be accepted as being occupationally related unless it were related at the very minimum to latency of exposure and possibly also to duration, and neither of these were the case.

Dr. Chase agreed on the shortcomings of the Yusho data but the three studies presented by Dr. Gaffey do have some shortcomings as well. They are not adequate to draw a definitive conclusion as to the carcinogenic risk of PCB exposure yet. There is also a question about Fischbein's study. According to Table 2 of Dr. Geffey's presentation, no dose relationship between liver function tests and PCB levels was found. The Fischbein article seemed to suggest to Dr. Chase that the main liver function tests were not significantly abnormal in the exposed group but that there was a statistically significant linear correlation between at least one of the liver function tests and PCB levels which has also been reported in other studies. Furthermore, in the 1979 Fischbein study it was stated that analysis of data to determine whether an association between PCB serum levels, cholesterol, and triglycerides existed would be reported at a later date.

Dr. John Brown of General Electric pointed out that chemically PCBs are lipophilic agents which tend to distribute at equal concentrations in all lipid pools in the body within a few days or weeks, and this means that the level in lipid reservoirs, such as the micellar lipids of the lipoproteins in blood tend to approach that of the PCB adipose tissue reservoirs. As a result, in any population of individuals having similar adipose tissue levels the blood level of PCB must of necessity parallel that of the level of blood lipid. So, there is a confounding in there. You predict that in a population of such individuals you would have to find a correlation unless the second law of thermodynamics were being violated. The correlation may become weak and difficult to see in a large population reflecting a large spread of adipose tissue PCB levels. The fact that there is a confounding between the levels of blood lipid and blood PCB means that it is difficult to establish correlations which infer that PCB has caused a change in lipid metabolism or a correlate of

elevated blood lipid such as hypertension. However, this does not mean that such hypothesized correlations cannot be tested. Instead, what it means is that the proper correlate to look for in testing either hypertension or serum lipid is not the serum PCB level but the adipose tissue PCB level.

In the case of the GE capacitor worker population, this has been explored by GE. A significant correlation of the serum PCB levels with both triglycerides and cholesterol was found. However, this correlation vanishes when correlations are made with adipose tissue PCB levels. Apparently Dr. Chase has made similar observations in his population and Dr. Brown raised the question whether this problem of confounding had been examined by others who are looking for abnormalities in lipid metabolism. It was pointed out by Dr. Kimbrough that to her knowledge, this had not been done. The fact that there are higher concentrations of lipids in serum might explain why some people have higher PCB blood levels. They were concerned about that in the Triene study because only blood levels were done. In a population that gets older, cholesterol levels seem to increase in people that eat a western type diet and with it, PCB blood levels. One argument against that would be the fact that there seems to be a relatively consistent ratio between PCB blood levels and PCB adipose tissue levels.

Dr. Brown indicated that to the extent these ratios have been examined by GE, a better correlation exists between adipose tissue PCB level and serum PCB level if a correction is made for the serum lipid level.

In response to a question by Dr. Friess, Dr. Kimbrough stated that there are some scientists who feel that it would be better to use adipose tissue for the determination of body burdens and as an exposure index. It is of course more difficult to get adipose tissue than it is to obtain blood samples. In large studies, the tendency has been to use serum and to determine PCBs in serum and assume that this represents a small portion of what is in adipose tissue. If results from populations are reviewed where laboratory analyses have been adequate, there seems to be a constant ratio between PCB concentrations in adipose tissue and in serum. This ratio varies with time to last exposure if exposure was recent and such problems as weight loss.

Subsequently Dr. Ian Webber of RTZ Corporation took issue with Dr. Caffey's statement that the Yusho oil incident was not a PCB related incident. Dr. Webber stated that in the case of the Yusho oil the PCBs had been in use for some considerable time and had degraded to give a "dirty" oil. It might be that the heat exchanger application of PCBs results in a worst case situation. Transformer oils on the other hand tend to degrade also. From a practical point of view, health studies that involve the largest amount of exposure to people in terms of either those getting rid of PCBs from contaminated oils or people involved with retrofilling transformers, should be done because these are the people who are likely to come into contact with dibenzofurans in PCB solutions. Have any studies been done with used PCBs?

Dr. Robert Bell from GE then informed the audience that they were in the middle of a project evaluating 40-year-old transformer fluid. Their findings to date indicate that the levels of dibenzofurans in 40-year-old fluid are at or below levels of the original PCBs that were delivered to GE. Thus, transformers operating at ambient temperatures do not pose a significant hazard.

Dr. Webber countered that those transformers operating at ambient temperature probably do not contain significant levels of dibenzofurans. There are however, a lot of factors in the use of PCB oils that come into play, the age, the amount of oxygen involved and hot spots caused by faulting.

Dr. Ball then mentioned the Binghamton case. Dr. Kimbrough asked whether everybody was familiar with the Binghamton situation. A transformer caught fire and a lot of combustion products were formed. Because the transformer also contained chlorinated benzenes, chlorinated dibenzodioxins formed in addition to chlorinated dibenzofurans, and because of the way the airshaft was situated in relation to the transformer, all of this material was evenly distributed over a very large state office building.

According to Dr. Ahmed, epidemiology is a two-edged sword and one can pretty much argue one's case depending upon how one interprets the data. Taking Dr. Gaffey's presentation and other information as well, a correlation is assumed between PCB blood concentration and actual exposure levels. In any kind of an epidemiologic study, it could be asked what the actual exposure level of the individual or cohort to a given agent was. Obviously, surrogates are used, and this could lead to all kinds of interpretations which may lead to positive correlations or negative correlations depending upon how it all comes out.

Secondly, to address the problem of confounding variables, Dr. Gaffey in one slide showed studies that were done since 1978. Some of the studies had been adjusted for confounding variables, and some of them had not. Dr. Kimbrough's data showed that PCB blood levels increase with age, at least in this particular group and that there was a sex difference in PCB blood level. This alone should warn us that we should try to adjust for variables in any of these studies. We have to adjust for age, ethnic differences, sex, and so on. Unless this is done for all studies, they cannot be compared on the same basis. Those studies that do take smoking habits and various other confounding variables into account are obviously far better. Unfortunately, many of the studies presented here have not looked into these factors properly. Dr. Gaffey made a suggestion that statistical power could be increased if studies were combined. Issue must be taken with pooling different populations from different geographical areas, such as the Brown study and the Bertazzi studies, which were done in two different parts of the world. The two populations obviously had ethnic differences and the study protocols used were quite different. Statistical power, even though we should look at all of the information in a qualitative way, has to be used in such a way that the statistician will agree gives a value of the sensitivity of the study. Some of the studies presented today were occupational and others nonoccupational. No information was given in the nonoccupational studies about the degree to which these individuals were exposed. Problems about latency periods exist. Since when and how long were individuals exposed to PCBs? How did their blood concentration correlate with their actual exposure years ago? There are so many problems with each of these studies, it cannot possibly be concluded whether or not PCBs cause the kinds of problems presented. We do have positive studies on dermatitis and chloracne. Then there are studies that conflict with one another. How can it be concluded that this means that there are no problems associated with PCBs?

Dr. Gaffey responded that he did not think that he said there were no problems, but that he specified the problems that seemed to be consistently turning up. In addition, there is almost no way to get around using surrogates for exposure in cohort mortality studies because it is not possible to measure with any precision the exposures that took place many years ago. Furthermore, confounding variables can frequently create spurious differences. They can rarely wipe out real differences because to create a difference where there, in fact, is none, any confounding variable will do. But to wipe out differences that exist, a conspiracy among a number of confounding variables is needed which is rather unlikely. The nonoccupational studies have in common the fact that their exposures are low, and the occupational studies have in common the fact that their exposures are high. It was not suggested that some simple piece of arithmetic can combine all these studies into one overall numerical estimate of power, but any reasonable assessment of the evidence has to regard not the power of an individual study, but the mass of the evidence, not necessarily in any formal way, although this could be done.

Dr. Kimbrough pointed out that individual susceptibility must be considered as well and that there seems to be an effect on reproduction in animals and that has not been studied to any great extent in humans. Animal data also suggest that females are perhaps more sensitive to the toxic effects of PCBs than males, and perhaps a greater effort should be made to look for health effects in females.

Dr. Brown responded to an earlier question raised by both Dr. Priess and Dr. Ahmed relating to the persistence of PCBs in the body and whether body burdens can be used as a measure of integrated exposure. They are attempting to answer this question through their studies of the GE capacitor worker group. The results to date indicate that most PCB isomers of the Aroclor 1254 type are highly persistent in the body, and they don't clear. Once they have been deposited in the body they are there forever unless they can be excreted through lactation. This may be true for a few isomers of the lower PCBs. There may be one or two isomers that can be used for tracking integrated exposure. This is not certain yet. Most of the lower chlorinated homologues are cleared but certainly the higher isomers represent a permanent record of exposure.

CHAPTER 6

HEALTH EFFECTS - LABORATORY STUDIES

The first paper in this chapter reviews the designs and results of animal studies performed to assess the toxicological effects of PCBs. For the sake of thoroughness, this review includes several studies completed prior to 1978. Data are presented and discussed on the following topics: skin effects, reproductive dysfunction, teratogenicity, fetotoxicity, liver structural and enzymatic alterations, gastric lesions, immunosuppressive effects, porphyria induction, carcinogenesis, and mutagenesis. The paper concludes with a summary of known effects in animals and a comparison of these to findings in humans.

The second deals specifically with one PCBs effect known to occur in both animals and humans -- liver enzyme induction. The data demonstrates in substantial detail how substitutions in the para, meta, and ortho positions can alter this activity. The enzyme induction by the PCBs is compared to the established structure-activity relationships of the phenobarbital and methylcholanthrene type inducers, as well as to the more hazardous polychlorodibenzo-p-dioxin compounds.

The discussion summary begins with a detailed analysis of the animal carcinogenicity studies, both those presented in the paper and several additional studies. The discussion summary then examines the "initiator-promoter" theory and the implications of rat liver nodules findings.

POTENTIAL HEALTH EFFECTS FROM EXPOSURE TO POLYCHLORINATED BIPHENYLS; LABORATORY STUDIES

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INTRODUCTION

This presentation is extracted from a recent review of potential health effects in the human from exposure to polychlorinated biphenyls (PCBs) and related impurities, as prepared for the National Electrical Manufacturers Association and the Edison Electric Institute under date of January 25, 1982. Two teams of specialists, representing the disciplines of medicine, toxicology, pharmacology, biochemistry and epidemiology, participated in the study. The first team reviewed the PCB health effects literature in detail through 1981, and prepared draft reports on the relationships between PCB exposure and specific health effects in test animals and in exposed people. The second team of scientists independently reviewed the draft material. Conclusions and opinions reflect contributions from both teams. The present focus is on effects in laboratory animal models subjected to controlled toxicological experiments, with additional comment on the bearing of these results on potential health problems in exposed human populations.

REPRODUCTIVE EFFECTS IN ANIMALS

Reproductive Problems

Numerous reports have appeared in the literature on the effects of polychlorinated biphenyls on reproduction in animals. Gellert and Wilson (1979) showed that female Sprague-Dawley rats given 30 mg/kg Aroclor 1221, 1242, and 1260 by gavage during days 14 through 20 of pregnancy had no effect on reproductive function as judged by (a) normal estrus cycles, (b) normal appearance of ovaries and uteri, and (c) fertility of males. Feeding 550 ppm of Aroclor 1254 for 67 days to Sherman rats resulted in fewer litters, smaller litter size, and 100% mortality by day three of the $F1_a$ pups. At 100 ppm survival of both $F1_a$ and $F1_b$ offspring was reduced. The pups were smaller than controls but appeared normal at weaning. Aroclor 1260 fed at a dietary level of 500 ppm (35.4 mg/kg) for 67 days prior to mating markedly reduced litter size and survival-to-weaning in the $F1_a$ and $F1_b$ generation. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations (Linder et al. 1974). A dietary concentration of 2.5 ppm of Aroclor 1248 produced alteration in the menstrual cycle of adult female rhesus monkeys. Menses were prolonged and menstrual bleeding was increased (Allen et al. 1979).

Keplinger et al. (1971) fed rats and dogs Aroclor 1242, 1254, and 1260 at doses of 1.0, 10.0, and 100 ppm. No adverse effect on reproduction was observed in rats given 1.0 and 10 ppm Aroclor 1242, but there was a decrease in survival of pups at 100 ppm of Aroclor 1242, 1254, and 1260 as well as a decrease in mating indices. The effect of PCBs on reproduction in beagle dogs

and swine has been studied by Earl et al. (1974). Aroclor 1254 interfered significantly with reproduction in dogs at doses above 2.5 mg/kg/day while in swine 10.0 mg/kg/day lowered fertility and survivability of neonates. The reproductive effects in dogs are questionable because of unexplained changes in the reproduction pattern among controls.

In a study reported by Allen et al. (1974), six adult, female rhesus monkeys were fed a diet containing 25 ppm of Aroclor 1248 for two months. During this period, all animals developed characteristic signs of toxicity, although they maintained a regular menstrual cycle. One animal died. At the beginning of the fifth month, or three months after discontinuance of exposure to the test substance, an attempt was made to breed the survivors. Three animals appeared to conceive, but only one succeeded in carrying her fetus to term. Although this infant was well developed, its body weight was considerably lower than that of the average rhesus infant. Examination of the tissues showed no gross or microscopic lesions.

In a second study by the Wisconsin group (Barsotti et al. 1976) involving 18 adult, female rhesus monkeys, nine were fed a diet containing 2.5 ppm, and nine were fed a diet containing 5 ppm of Aroclor 1248. After seven months on these diets, the eight surviving animals from the 2.5 ppm group and eight from the 5 ppm group were mated with control males. All animals in the 2.5 ppm group became pregnant, but three of these resorbed their embryos; the remaining five gave birth to live infants. In the 5 ppm group six animals became pregnant. These impregnations resulted in three abortions, one resorption, one stillbirth (suffocation during difficult delivery), and one uncomplicated birth. At birth, the six infants were small; however, other than their small stature and focal areas of dermal hyperpigmentation, their general appearance, hemograms, and osseous development as evaluated radiographically were normal.

Teratogenesis

Mice

PCBs were not teratogenic in mice at dosages up to 500 mg/kg given on days one through six or days seven through 11 of gestation (Toeruek 1973). However, a recent study (Marks et al. 1981) with the hexachlorocongener 3,3', 4,4', 5,5'-hexachlorobiphenyl in mice at dosages in the range of 0.1-16 mg/kg/day during days six through 15 of gestation produced a significant increase in fetal malformations, a significant decrease in average fetal weight, and an increase in the percentage resorptions.

Some mice exposed to 3,4,3'4'-tetrachlorobiphenyl were reported to exhibit a "waltzing syndrome" (Tilson et al. 1979). The dosage was 32 mg/kg administered by gavage to the mothers on days 10 through 16 of gestation. The syndrome consisted of increased motor activity, hyperreflexia, and episodes of head bobbing and rotational movements that were present up to at least eight months of age. Not all exposed mice were affected similarly, but even those that did not exhibit the syndrome showed diminished performance in various neurobehavioral tests. Tilson et al. (1979) refer to these observations as the "behavioral teratology" of 4-CB.

Rats

There are a plethora of studies in which the reproductive effects of feeding various Aroclors to rats have been investigated (Calandra 1976, Gellert and Wilson 1979, Keplinger et al. 1971, Keplinger et al. 1972, Linder et al. 1974, Villeneuve et al. 1971a). Collectively, these studies reported fetotoxicity as the dosage was increased, but no teratogenicity was demonstrated. It may be useful to note the order of magnitude of the dosages involved. Villeneuve et al. (1971a) found no effect from the administration of dosages of up to 100 mg/kg per day orally to pregnant females from the sixth through the fifteenth day of gestation.

Dogs

In the study conducted by Earl et al. (1974), pregnant bitches were given dosages of 0.25, 1.0, or 5.0 mg/kg of Aroclor 1254 from the day of breeding to the day on which they were necropsied. At 5.0 mg/kg, there was an increase in the percentage of resorptions, a decrease in the number of live pups per litter at birth, and a reduction in the percentage of those surviving to two weeks. The terata observed consisted of enlarged fontanelles, cleft palates, and supernumerary phalanges. This dosage was said to limit diet consumption severely. The authors state that one litter of the controls "may have had a genetic defect that caused an unusually high incidence of terata."

Swine

Earl et al. (1974) included miniature swine (Hormel strain) in the investigation described immediately above. Pregnant sows were given daily oral dosages of 1 mg/kg, 10 mg/kg, or 30 mg/kg of Aroclor 1254. Dosing began 21 days before breeding and was continued until the day of necropsy. The authors concluded that dose-related effects were seen at all treatment levels as evidenced by decreases in the number of pregnancies, number of live pigs farrowed per litter, and percentage of live offspring after two weeks of age. Syndactyly and cleft palates were observed in the young of sows receiving 10 mg/kg, and patent fontanelles and cleft palates in the offspring of those receiving 30 mg/kg. As in the case of the dogs, the higher dosages caused a marked reduction in food consumption.

Fetotoxicity

Rabbits given 1.0 mg/kg of Aroclor 1254 daily for 28 days of gestation showed no effect on the developing fetus but doses of 12.5 to 50 mg/kg were fetotoxic. Rats appear to be more resistant than rabbits, as doses up to 100 mg/kg do not cause fetal deaths or malformations (Villeneuve et al. 1971a) Embryotoxic effects of Kanechlor 300 and 500 were produced in Sprague-Dawley JCL rats when fed at levels of 500 ppm in the diet throughout gestation (Shiota 1976b). Diets containing 5.0 ppm of Aroclor 1248 were fetotoxic to rhesus monkeys. Studies in mice on the transfer of PCBs to fetuses and offspring indicate that the amount transferred depends upon the chemical structure of the individual compounds and the position of the chlorine atoms within the chemical structure (Masuda et al. 1978, 1979).

CARCINOGENICITY

Rats

The three major studies pertaining to PCBs and hepatic carcinoma in the rat are those of Kimbrough et al. (1975), Calandra (1975), and the National Cancer Institute (1978). Kimbrough and coworkers, using the Sherman strain of rat, found that 100 ppm of Aroclor 1260 in the diet for 21 months produced a significant increase in the incidence of hepatocellular carcinoma in female rats. Male rats were not studied.

The administration of three different Aroclors at a dose of 100 ppm to rats for 24 months did not induce hepatocellular carcinoma (Calandra 1975). The author states that this negative finding was based on evaluation of the liver sections by three pathologists, who read the slides independently and separately. He states further that Professor P. Pour reevaluated the Kimbrough slides and did not agree with the reported findings.

Results of the National Cancer Institute (1978) bioassay of Aroclor 1254 for possible carcinogenicity differ from those of Kimbrough with Aroclor 1260. Fischer 344 rats were used and the PCB mixture was administered at three dose levels (25, 50, and 100 ppm) in the diet for 104-105 weeks. The effect of the treatment on body weight and mortality at the higher dose levels demonstrated that maximum tolerated doses were used, thus providing a satisfactory test of carcinogenic potential. A sufficient number of rats of both sexes was available for meaningful statistical analyses of the incidence of late-developing tumors. Treatment was associated with only three hepatocellular carcinomas in male rats, which was not significantly different from the controls, and it was concluded that Aroclor 1254 was not carcinogenic in the bioassay.

Ito et al. (1974) treated rats with different doses of Kanechlor 300, 400, and 300 for periods up to 52 weeks; hepatocellular carcinomas were not observed, but the duration of treatment was probably too short to rule out the possibility of a positive response.

Mice

Ito et al. (1973) observed hepatocellular carcinomas in mice only in the high dose group given Kanechlor 500; lower doses of Kanechlor 500 or the other Kanechlors did not produce a carcinogenic response. The administration of 300 ppm of Aroclor 1254 in the diet for 11 months did not induce hepatocellular carcinomas in mice (Kimbrough and Linder 1974).

Dogs

Hepatocellular carcinoma did not occur in dogs (four male and four female per group) receiving 1, 10, or 100 ppm of Aroclor 1242, 1254, and 1260, respectively, in their diets for two years (Calandra 1975).

General Comment

Animal studies do not provide convincing evidence that PCBs induce liver cancer. Of the major studies in the rat, one has been judged positive and two

have been negative. Hepatic carcinoma was not produced in the dog. Chronic administration of Aroclors in the rat did not induce bladder cancer, gastrointestinal carcinomas, or cancer of the thyroid gland, pituitary gland, adrenal gland, uterus, lung, hematopoietic system, or other organs. Data from the mouse provide only limited and restricted evidence for a carcinogenic effect of the Japanese product Kanechlor 500.

MUTAGENIC EFFECTS

Tests for mutagenic activity of PCBs in mammalian cell systems or intact animals were uniformly negative. Specific tests and their results are listed below.

Cytogenetic Analysis In Vitro

Hoopingarner et al. (1962) activated cultured human lymphocytes with phytohemagglutinin and treated them with 100 ppm of Aroclor 1254 at various stages of a division cycle. The cells then were examined for chromosomal aberrations for the different stage treatments. Aroclor 1254 had no apparent effect on chromosomal integrity as measured by cytological evidence.

Dominant Lethality in Rodents

Green et al. (1975b) concluded that Aroclors 1242 and 1254 were not mutagenic since they failed to induce dominant lethal mutations in rats. In similar studies reported both by Keplinger et al. (1972) and Calandra (1976), Aroclors 1242, 1254, and 1260 were administered to male, albino mice in a single intraperitoneal dosage of either 500 mg/kg or 1000 mg/kg. Subsequent matings of these animals to control females showed no effect of treatment on the number of implantation or resorption sites, number of viable embryos, or preimplantation loss.

Ames Test in Salmonella

Aroclors 1221, 1254, and 1260 showed little mutagenic activity in the Ames test (Wyndham et al. 1979) with indications that Aroclors with lower chlorine contents are weakly positive in the Salmonella test systems. However, these results must be discounted since they could not be replicated (McMahon et al. 1979, Safe 1980). As a general finding, Heddle and Bruce (1977), McMahon et al. (1979), Schoeny et al. (1979), and Safe (1980) were unable to find evidence of mutagenicity of PCBs in bacterial test systems.

Cytogenetic Analysis In Vivo

In two investigations, PCBs were judged not to have produced chromosomal abnormalities. Dikshith et al. (1975) examined seminiferous tubules of rats at different intervals after daily dosages of 50 mg/kg of Aroclor 1254. Green et al. (1975) examined both bone marrow and spermatogonial cells of rats after either a single dose of 5000 mg/kg of Aroclor 1242, or four daily doses of 500 mg/kg. In all cases, it was concluded that no significant chromosomal damage had occurred.

Micronucleus Test

The micronucleus test is a test for agents that tend to break chromosomes (clastogens). A micronucleus is a fragment of chromatin that has broken away from a chromosome during cell division and has failed to be included in either of the daughter nuclei. The phenomenon can be observed best in newly formed erythrocytes since these stain differently from the mature erythrocytes in that they exhibit polychromasia for a period of 24 hours or so. Heddle and Bruce (1977) reported Aroclor 1254 as negative in a micronucleus test.

OTHER HEALTH EFFECTS

Effects on Skin

Epithelial and follicular hyperplasia and hyperkeratosis have been reported to occur following the application of PCBs on the skin of rabbits (Vos and Beems 1971). Skin lesions have also been observed in rats and guinea pigs from the application of PCBs. Repeated application of Aroclor 1260 to the skin of rabbits caused thickening of the skin as a result of hyperplasia and hyperkeratosis of the epithelium (Vos and Beems 1971).

Cutaneous effects have been elicited in male rhesus monkeys fed a diet containing 300 ppm Aroclor 1248. Within one month the animals lost considerable hair from the head, neck, and back (Allen et al. 1973, 1975). Similar effects have been observed in female rhesus monkeys fed a diet containing 25 ppm Aroclor 1248 for two months (Allen et al. 1974). Within six weeks the animals began to lose hair and developed obvious signs of edema of the lips and eyelids. Small pustules involving hair follicles appeared about the mouth, cheeks, and neck. Abrahamson and Allen (1973) showed that the infant monkey was able to tolerate doses of PCBs that produce extreme morbidity in adult monkeys. They suggested that there may be variations in absorption, distribution, metabolism, storage, and excretion in adult and infant monkeys that may account for these differences.

Effects on Liver

Liver Weight

The administration of PCBs may induce an increase in liver weight. The effect is more prominent at the higher dose levels; the lower doses of PCBs do not increase liver weight. The detailed data of Kimbrough et al. (1972) include findings that Aroclor 1260, administered with the diet in doses of 500 and 1000 ppm for eight months, significantly increased liver weight in male and female rats; doses of 20 and 100 ppm were effective only in male rats. Aroclor 1254 increases liver weight in both male and female rats at doses of 20, 100, and 500 ppm.

The increase in liver weight is correlated with hepatic cell hypertrophy and an increase in smooth endoplasmic reticulum in the rat, rabbit, and monkey (Vos et al. 1972, Allen and Abrahamson 1973, Bruckner et al. 1974, Allen, Norback and Hsu 1974, Allen, Carsteus, and Barsotti 1974).

General Histology

The administration of PCBs to experimental animals will produce histopathological changes in the liver. The main effects obtained are the production of enlarged hepatocytes, fat droplets, and slight degree of necrosis; the occurrence of these changes depends particularly on the dose of the PCB and to some extent on the particular Aroclor. Necrosis seems to be more severe in the rabbit than in the rat. In general, the morphological changes observed in the liver of PCB-treated animals are similar to those found after treatment with other chlorinated hydrocarbons.

In a detailed study, Kimbrough et al. (1972) found that Aroclor 1260 (20, 100, 500, and 1000 ppm) and Aroclor 1254 (20, 100, and 500 ppm), given in the diet of rats for eight months, produced enlarged liver cells and cytoplasmic inclusions. At the higher doses, there was evidence of lipid accumulation, which was associated with a foamy cytoplasm, and pigment accumulation in the Kupffer cells.

Adenofibrosis

Adenofibrosis (synonyms: bile duct proliferation, bile duct adenomatosis, cholangiofibrosis, fibroadenoma) has been observed in some rats receiving PCBs. Adenofibrosis is generally regarded as a benign lesion. Kimbrough et al. (1972) observed adenofibrosis in rats treated with Aroclor 1260 and 1254; the effect was observed at the higher dose levels and particularly in animals receiving Aroclor 1254. When the feeding of 500 ppm of Aroclor 1254 was discontinued and the animals studied up to 10 additional months, the fat and liver content of PCB remained high and the adenofibrosis persisted (Kimbrough et al. 1973). In a later paper involving treatment with Aroclor 1260 at 100 ppm for 21 months, mention is only made that a few rats showed areas of adenofibrosis, indicating a low degree of response, but data are not tabulated (Kimbrough et al. 1975). In further studies by the same group adenofibrosis was not observed in rats fed Aroclor 1016 or 1242 (100 ppm) for up to 10 months (Burse et al. 1974).

The study of the National Cancer Institute (1978) did not reveal adenofibrosis in rats receiving Aroclor 1254 at doses of 25, 50, and 100 ppm for 104-105 weeks.

Hyperplastic Foci and Nodular Hyperplasia

Hyperplastic foci or hyperplastic areas represent minimal changes in liver hepatocytes. Such foci or areas of hyperplastic changes are uncommon in untreated young rats, but increase with age. The hyperplastic areas or foci may coexist with nodular hyperplasia and/or hepatocellular carcinoma. The significance of hyperplastic foci is that they may be part of a spectrum capable of progressing to a nodule (Squire and Levitt 1975).

Keplinger et al. (1971) did not observe hepatic lesions in rats receiving different Aroclors for 18 months; however, reevaluation of the slides indicated a significant increase of nodular hyperplasia in the treated animals (see National Cancer Institute 1978).

Ito et al. (1974) observed nodular hyperplasia in rats receiving 100, 500, or 1000 ppm of Kanechlor 500; a lesser effect was observed with Kanechlor 400 and there was no significant effect of Kanechlor 300.

Kimbrough et al. (1975) reported a significant increase in the incidence of hyperplastic foci or areas and neoplastic nodules in female rats given 100 ppm of Aroclor 1260, mixed with the diet for 21 months. In contrast, in another study, the dietary administration of 100 ppm of Aroclor 1242, 1254, and 1260 for 24 months increased the incidence of nodular hyperplasia; a lesser effect was obtained with 10 ppm, and 1 ppm did not produce a positive response (Calendra 1976, Levinskas 1981). All three Aroclors produced an increased number of hepatomas at the high dose. There was no evidence of metastasis or invasiveness, and the lesions were regarded by the pathologists as benign tumors.

Gastric Lesions

The oral administration of PCBs to nonhuman primates has been shown by Allen and coworkers to induce hypertrophy and hyperplasia of the gastric mucosa. Mucosal cysts may be present within the epithelium of the stomach, but in the mucosal lining and the submucosa, there may be edema of the submucosa of the stomach. In addition, there is frequently invasion of the underlying mucosa by isolated glandular elements of the mucosal epithelium. General effects of this nature have been produced in the monkey by:

1. A single oral dose of 1.5 g or 3 g of Aroclor 1248 (Allen, Norback, and Hsu 1974).

2. Aroclor 1248, 300 ppm and Aroclor 5460 (a polychlorinated triphenyl), 5000 ppm in the diet for 90 days (Allen, Abrahamson, and Norback 1973, Allen and Norback 1973).

3. Aroclor 1248, 25 ppm in the diet for 2 months (Allen, Carstens, and Barsotti 1974).

4. Aroclor 1248, 100 ppm in the diet for 2-3 months (Allen 1975).

5. Aroclor 1248, 2.5 ppm and 5.0 ppm in the diet for up to one year (Barsotti and Allen 1975). (Gastric changes were not listed for these doses in Allen's review.)

6. The hyperplastic gastritis may persist for over one year following discontinuance of PCB exposure (Allen 1975).

A mixture of low chlorinated biphenyls (Clophen A-30) was not observed to produce gastric lesions in the monkey (Ieiropoulos et al. 1977).

Hypertrophy and hyperplasia of the gastric mucosa have not been reported in the rodent (Allen and Abrahamson 1973), rabbit (Vos 1972), or in other species (Vos and Koerman 1970).

Enzyme Induction

Various studies have reported on the effect of short term exposure of rats to mixtures of chlorinated biphenyls. One such study by Ecobichon et al. (1974) used the Aroclors identified as Aroclor 1016, 1221, 1242, and 1254, and another study by a French investigator, Narbonne (1980), used a French preparation known as Phenoclor DP6. In both studies the sample material consisted of congeners of the chlorinated biphenyls. These studies showed that when rats were administered 10 ppm of the PCBs in their diet for only one day, the livers from the animals showed induction of P-450, aniline hydroxylase, and aminopyrine-N-demethylase. The induction of these enzymes was maximum in about five days. If the rats were given 10 ppm of the PCBs in the diet for eight consecutive days, induction occurred in three to five days and to a lesser extent over the remaining eight-day interval. In the Ecobichon study, intraperitoneal injection of the Aroclors in doses of 50 mg/kg was made for three consecutive days. Animals were sacrificed 96 hours later. Mixed function oxidative enzymes were found to be induced in the liver. The greatest induction was seen with the more highly chlorinated Aroclors. The enzyme induction occurred rapidly on exposure to the Aroclors, and particularly to those Aroclors that are more highly chlorinated. The PCBs used in these studies were commercial grade.

Aroclors 1242 and 1254 are potent inducers of hepatic microsomal enzymes. A single intraperitoneal injection of 100 mg/kg of Aroclor 1242 to rats increased liver weight, total microsomal activity, as measured by hydroxylation of acetanilide and N-demethylation of aminopyrene, and hepatic cytochrome P-450 (Bruckner et al. 1973).

Immunosuppression

Environmental chemical effects on immunocompetence have been extensively reviewed by Faith, Luster and Vos (1980). A number of studies cited by them found PCBs to be immunosuppressive in various species (from ducklings and chicks to mice and monkeys), in some cases at dose levels that produce little effect other than hepatocyte hypertrophy. Only one study (Street and Sharma 1975), which involved feeding low levels (0.18 to 6.3 mg/kg/day) of Aroclor 1254 to rabbits, showed no significant effect on humoral or cell mediated response. Our review of the Street and Sharma report indicated that there was a dose-related trend toward immunosuppression in their animals and at the higher doses (2.1 and 6.3 mg/kg/day) for four to eight weeks the animals showed significant increase in liver weight. The Faith, Luster, and Vos review lists several general factors (nutritional status, hormonal levels, and amounts of immunoregulatory proteins such as alpha-fetoprotein) that influence immune function. Therefore those studies in which immune function was studied following exposure to levels of the PCBs that resulted in overt toxicity are meaningless from an immunotoxicologic viewpoint. In contrast, immunosuppression at dosage levels that do not produce general toxicity would be significant.

Porphyria

The administration of PCBs to rats will produce a delayed type of porphyria, i.e., deposition of porphyrins and their degradation products in tissues and excreta. Although the mechanism of action of the PCBs is unknown,

it apparently differs from that of other porphyrogenic compounds such as hexachlorobenzene or isopropylacetamide. It has been thought that the effect of PCBs in animals may indicate that these compounds can induce conditions such as porphyria cutanea tarda in man, but clinical data have not shown this to occur in PCB-exposed workers.

The fecal content of coproporphyrin and protoporphyrin of rabbits was increased by Aroclor 1260 and by hexachlorobiphenyl, but the difference was statistically significant only for coproporphyrin (Vos et al. 1972). Bruckner et al. (1974) observed an increased excretion of urinary coproporphyrin in rats given Aroclor 1242, 5 or 25 ppm for two, four, and six months.

Goldstein et al. (1974) emphasized the delayed onset of the PCB-induced porphyria; rats fed 100 ppm of Aroclor 1254 became porphyric after two or seven months of treatment. Although the excretion of coproporphyrin and other porphyrins was increased, the largest elevation was in the uroporphyrin fraction. There was also a marked accumulation of uroporphyrin in the liver. The studies of Goldstein et al. (1974) suggest that PCBs may effect uroporphyrin formation or utilization. The induction of delta-aminolevulinic synthetase (ALA synthetase, a rate-limiting enzyme in heme synthesis) does not appear to be the mechanism by which porphyria is induced by PCB, as it is for many porphyrogenic chemicals. In their studies the increase in ALA synthetase activity was probably secondary to the porphyria. Also, Aroclor acts to increase liver cytochrome P-450 rather than to decrease it.

Hexachlorobenzene is known to induce a delayed type of hepatic porphyria similar to that produced by Aroclor 1254. However, the two responses apparently differ significantly as Goldstein et al. (1974) reported that the rats fed PCBs did not exhibit the nervous or cutaneous signs associated with hexachlorobenzene poisoning.

GENERAL COMMENT, SUMMARY AND OPINIONS

Body Burdens, Metabolism, and Kinetics

Some general observations derive from consideration of the data on the storage, distribution, metabolism and excretion of commercial PCB mixtures (with associated impurities such as the PCDFs) in mammalian systems. The dynamics of the pathways by which these processes occur in test animal models have been explored intensively and have been fairly well worked out. The variability of PCB effects with species of test animal models has been investigated for a number of important effects, and some general structure versus activity rules have emerged from these studies. There thus exists a conceptual framework, but not experimental data, for describing such phenomena in humans. Further, important data on the biochemical interactions of PCBs, PCDFs and metabolites with tissues have been obtained that bear directly on interpretation of toxic effects in these tissues.

General Toxicity

From acute, subchronic and chronic studies of PCB effects in test animal models, a general summary of health effects encountered includes: (a) a low order of acute toxicity; (b) skin lesions; (c) effects on liver tissue that

are generally reversible at lower doses but that trend toward irreversibility with increased dosing; (d) effects on the gastric mucosa; (e) effects on the menstrual cycle and on reproduction; (f) porphyria; (g) effects on the kidney; and miscellaneous changes including hematologic alterations, thymic atrophy and lymphatic changes.

There is a considerable range of species susceptibility to biological activity of the PCBs, with one representative comparison of order of decreasing activity being mink, monkey, rat. It is concluded that there is no basis for determining which species most accurately serves as a model for prediction of general subchronic/chronic toxicity in man; rather, the judgment of the most suitable surrogate for man must be made independently for each major health effect.

Skin and Other Cutaneous Tissues

Commercial PCBs are capable of producing skin lesions in the monkey model and in exposed humans. Chronic administration of PCBs to the monkey produces chloracne. In humans occupationally exposed to PCBs skin disorders including chloracne have occasionally been observed. These skin disorders have been shown to be reversible in some of the animal and human studies.

Liver Effects

PCB mixtures are capable of generating important effects on liver tissue in animal models. These effects include: (a) increase in liver weights; (b) histopathologic changes in liver including enlarged hepatocytes, deposition of fat droplets in tissue, and tissue necrosis and cell death; (c) enlargement of the liver; (d) the occurrence of adenofibrosis, a benign lesion; and (e) an increase in proliferative lesions of the liver, including increases in hyperplastic foci and nodular hyperplasia. These increases in proliferative lesions appear to increase in severity with increasing degree of chlorination of the PCB mixture, as seen in the greater potency of Aroclor 1260 over Aroclor 1254. The effects on liver tissues are generally reversible at lower doses but trend toward irreversibility with increased dosing.

Gastric Lesions

When tested in monkeys and rodents, PCBs show species specificity with respect to a toxic effect on the gastric epithelium, or stomach lining. In the monkey, with Aroclor 1242, there is a mucous conversion of the gastric epithelium that can best be described as a dysplastic growth pattern. This growth pattern does not constitute a neoplastic transformation. In rodents, PCBs do not evoke this effect on the gastric mucosa.

Carcinogenesis

A sizeable volume of animal experimental work has been directed toward chronic studies in several species: the mouse, the rat and the dog. There are some areas of interpretation that are still under debate by the investigators concerned, but in the opinion of the Category I team the following represents conclusions that can be drawn from presently available data: (a) the evidence on carcinogenicity is negative for gastrointestinal carcinoma and bladder

carcinoma in rats and hepatocellular carcinoma in the dog; (b) some studies have reported an increase in hepatocellular carcinoma in mice and rats exposed to commercial PCBs chronically, whereas other studies have afforded negative results.

Reproductive Effects

Experimental work with commercial PCB mixtures and animal models has been directed to the reproductive process itself, to teratogenesis in the offspring, and to possible fetotoxicity. In most test species PCBs produce deleterious effects on reproduction at high dosages. In the female monkey at relatively high dosages, difficulties are encountered in conception, in implantation of the fertilized ovum, and in carrying the fetus to term. Similarly dosed, the male monkey does not transmit these difficulties to the reproductive process.

The potential for PCB-induced teratogenesis, or production of defects in the embryo or fetus, has been investigated in several species of test animals. Most animal tests have yielded negative results when PCBs were administered to pregnant females during the critical periods for organogenesis. Several possible exceptions to this statement might be noted. In mice, a single congener, 3,4,3',4'-tetrachlorobiphenyl, induced a behavioral defect ("waltzing syndrome") that may be related to an anatomical defect in the inner ear. PCBs in mice may produce some delay in implantation. In rats, no gross teratological changes were observed, but some alterations in thyroid structure or function produced by PCB administration might fall under the definition of terata. In dogs and in swine, no teratological effects were noted at lower PCB doses; however at the highest doses of PCB in the feed, at which the dam suffers marked reduction in food consumption and is therefore nutritionally deficient, there are dose-related teratological effects in the offspring. In monkeys dosed with PCBs, there are no dose-related abnormalities in the offspring, but they are smaller in size.

In test animals, i.e. rats, dogs and rabbits, PCBs are fetotoxic when administered at relatively high doses to the pregnant female.

Immunocompetence

With respect to potential PCB effects on immunocompetance in test animal systems, some observations are pertinent to the assessment of probable hazard in the human. It has been observed in chicks that if the PCB dosages are high enough, atrophy of the splenic pulp and necrosis of the lymphoid system can be demonstrated. Similar indicators of change in immunocompetence can also be produced in ducklings, mice, and monkeys as a consequence of severe change in nutritional status. High doses of PCBs leading to marked reduction of food intake and utilization can lead to such changes in nutritional status. These considerations lead to two general conclusions relative to the PCB-exposed humans: (a) as projected from animal studies, if the PCB dosage is high enough to lead to general toxicity in the human (e.g., decreased food intake, fall in body weight, fall in weight gain), immunosuppression may be demonstrable secondary to malnutrition; and (b) at lower dose levels, there is no likelihood of significant immunosuppression.

Porphyria

Porphyria, or deposition of porphyrin-derived pigments in liver and urine, has been observed in experimental animals such as the rat and rabbit dosed with PCBs. The effect has a delayed onset, and especially in the rat, seems to take place by mechanisms different from those employed by known chemical porphyria-producers in the human.

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PCBs: STRUCTURE-ACTIVITY RELATIONSHIPS

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are readily synthesized by the direct chlorination of biphenyl using iron salts and/or iodine as the catalyst. The degree of chlorination of the resulting mixture is controlled by the relative amount of chlorine used in the reaction and most commercial products are graded and sold by their weight per cent chlorine content (Hutzinger et al. 1974). PCBs have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, flame retardants, organic diluents and dielectric fluids. The unusual industrial versatility of PCBs is directly related to their physical properties which include: a) resistance to acids and bases, b) compatibility with organic materials, c) resistance to oxidation and reduction, d) excellent electrical insulating properties, e) thermal stability and f) non-flammability. Unfortunately, these physical properties coupled with their widespread use, relatively low acute toxicity and improper disposal has resulted in the contamination by PCBs of every component of the global ecosystem (Hutzinger et al. 1974, Landrigan 1980, Jensen and Sundstrom 1974, Wassermann et al. 1979, Fujiwara 1975). Moreover, the lipophilic nature and persistence of PCBs also contributes to their high bioaccumulation potential and their biomagnification in higher trophic levels of the food chain (Safe 1980). PCBs residues are routinely detected in fish, wildlife and human adipose tissue, blood and breast milk.

COMMON ANALYTICAL AND TOXICOLOGICAL PROBLEMS

The analysis of PCB residues in environmental samples present numerous problems which include the following:

1. PCB formulations and environmental isolates are highly complex mixtures of isomers and congeners and

2. the composition of PCBs obtained from diverse environmental matrices are highly variable due to the different rates of biodegradation, vaporisation, photodegradation and chemical degradation of the individual PCB isomers and congeners (Safe 1980, Ballschmiter et al. 1978, Harvey and Steinhauer 1974).

There are 209 possible PCB isomers and congeners and most of the commercial mixtures and environmental samples exhibit a multitude of peaks and this is particularly evident in the capillary gas chromatogram of the commercial PCB, Aroclor 1260. It has been common practice to quantitate environmental PCBs by comparing their packed column gas chromatograms with the patterns exhibited by known amounts of individual commercial PCBs or mixtures of these formulations (Sawyer 1978, Albro et al. 1981). This method relies on pattern matching using specific peaks for quantitation. It is clear that if the gas chromatograms of the environmental PCB residues cannot be "matched" with an appropriate cocktail containing known amounts of the commercial formulations then quantitation is not possible. Comparable analytical problems have been encountered in the analysis of other complex halogenated aromatic pollutants, and polynuclear aromatic hydrocarbons (NRCC 1981). These problems experienced in PCB analysis by gas chromatography (GC) can be resolved in the following way:

1) The samples must be analyzed by high resolution capillary gas chromatography to ensure the maximum separation of the isomers and congeners,

2) all 209 PCB isomers and congeners must be available as analytical standards for the identification and quantitation of the individual components in the analyte.

In collaboration with Dr. M. Mullin and several coworkers, considerable progress has been made on the synthesis and characterization of all 209 PCBs (Table 1) and this has resulted in the positive identification of the individual PCB components present in commercial mixtures and in environmental samples. Based on the numbering scheme proposed by Ballschmitter and Zell (1980), Figure 1 illustrates the identification of the individual PCBs in Aroclor 1260 using the synthetic PCB standards (Mullin et al. 1981).

Most reviews discussing the biologic and toxic effects of commercial PCBs note that the relative potencies of these formulations depends on their degree of chlorination and also on the species being used in the study (Ecobichon and Comeau 1974, Kimbrough 1974). The results clearly suggest that since the toxicities of the mixtures are variable, then the activities of the individual components of these mixtures may also be different. Research on the comparative biologic and toxic effects of PCBs has clearly illustrated the marked differences in the comparative toxicities of PCB congeners as noted below.

1) 2,3',4,4',5-Pentachlorobiphenyl is highly embryotoxic to chickens whereas 2,2',5,5'-tetra and 2,2',4,4',5,5'-hexachlorobiphenyl are relatively non-toxic (Ax and Hansen 1975).

2) 3,3',4,4'-Tetrachlorobiphenyl is highly toxic to Rhesus macaques whereas the 2,2',5,5'-isomer elicits no clinical effects (McNulty et al. 1980).

3) The toxic lesions caused by 3,3',4,4',5,5'-hexachlorobiphenyl in male mice were much more severe than those observed after administration of the 2,2',4,4',6,6'-, 2,2',4,4',5,5'- and 2,2',3,3',6,6'-hexachlorobiphenyls (Biocca et al. 1981).

4) A comparison of the toxicity of 2,3,3',4,4'-penta- and 2,3',4,4'-tetrachlorobiphenyl showed the former congener to be highly active and the latter compound to be inactive (Yamamoto et al. 1976).

5) Chronic administration of 2,2',3,3',4,4'- and 2,2',3,4,4',5'-hexachlorobiphenyl caused an accumulation of liver porphyrins whereas administration of the 2,2',4,4',5,5'-hexachlorobiphenyl had no effect on hepatic porphyrin levels (Stonard and Grieg 1976).

6) 3,3',4,4',5- and 2,3',4,4',5-pentachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl cause thymic atrophy in rats whereas the 2,2',3,3',4,4',5- and 2,2',3,4,4',5,5'-heptachlorobiphenyls were not toxic (Yoshihara et al. 1979).

Table 1. Progress in the Synthesis of PCB Isomers and Congeners

Cl Groups	1	2	3	4	5	6	7	8	9	10	Total
No. of Isomers	3	12	24	42	46	42	24	12	3	1	209
No. Synthesized or Available	3	9	18	40	42	40	24	12	3	1	192

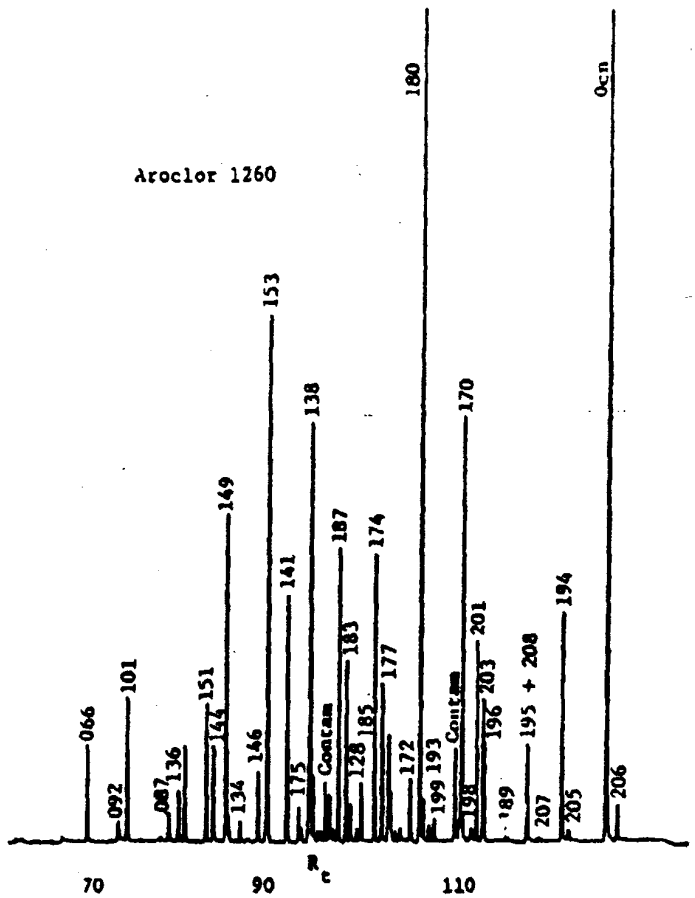


Figure 1. High resolution glass capillary gas chromatogram of Aroclor 1260.

It is evident that subtle changes in the structure of PCBs can result in marked differences in toxicities. Thus, an assessment of the potential long term effects of PCBs on biological systems will depend on the concentration of the individual toxic congeners which are present and which persist in the animal species. This necessitates not only a high resolution quantitative and qualitative analysis of PCB residues, but prior knowledge concerning the potential or actual toxicities of the individual PCBs.

PCBs AS INDUCERS OF HEPATIC MICROSOMAL DRUG-METABOLIZING ENZYMES

One of the most characteristic biologic effects of the commercial PCBs is their induction of numerous hepatic microsomal drug-metabolizing enzymes in rodents and particularly the cytochrome P-450-dependent monooxygenases (Ecobichon and Comeau 1974, Alvares and Kappas 1977a,b, Alvares et al. 1973, Ryan et al. 1979). Administration of these mixtures results in increases in microsomal aniline hydroxylase, benzo(a)pyrene hydroxylase, biphenyl hydroxylases, halobiphenyl hydroxylases as well as numerous N- and O-dealkylases. The potent induction of these cytochrome P-450 dependent monooxygenases by Aroclor 1254 has resulted in the widespread use of PCB-induced microsomes as the source of metabolic activation for chemicals in the Ames test for their bacterial mutagenicity (Hollstein et al. 1979).

Inducers of microsomal monooxygenases are classically subdivided into two main categories (Conney 1967, Lu et al. 1976, Sladek and Mannering 1976). One class is typified by phenobarbitone (PB) and the other by 3-methylcholanthrene (MC). PB-type inducers enhance microsomal N-demethylase (2-3-fold), NADPH cytochrome P-450 reductase (2-3-fold) biphenyl-4-hydroxylase (2-4-fold) and aldrin epoxidase (3-5-fold) whereas MC-type inducers enhance microsomal benzo(a)pyrene hydroxylase (8-12-fold), 4-chlorobiphenyl hydroxylase (8-10-fold), ethoxyresorufin O-deethylase (up to 30-fold) and biphenyl 2-hydroxylase (8-12-fold). The preferential induction of specific monooxygenases by MC- and PB-type inducers coupled with the different electrophoretic and spectral properties of their induced microsomal enzyme preparations clearly differentiates between the two classes of inducers. The enzyme induction properties of Aroclor 1254 and other commercial PCBs do not resemble that of either PB or MC but are similar to the pattern produced after co-administration of PB + MC (Alvares and Kappas 1977a,b, Alvares et al. 1973). More recent studies have shown that Aroclor 1254 induces cytochromes P-450b and P-450c which are also the major isozymes induced by PB and MC respectively (Ryan et al. 1979). The mixed-type induction activity of Aroclor 1254 was presumed to be due to the individual PB- and MC-type inducers present in this mixture.

PCBs AS MICROSOMAL MONOOXYGENASE ENZYME INDUCERS: EFFECTS OF STRUCTURE ON ACTIVITY

PB-Type Inducers

Initial studies suggested that PCBs which are PB-type inducers must contain at least two para and two ortho substituents; any further substitution changes only the quantitative nature of the induction activity (Poland and Glover 1977, Goldstein et al. 1977). There is ample evidence for these correlation and some of the most active PB-type inducers are 2,2',4,4'-tetra-, 2,2',4,4',5,5'hexa- and 2,2',3,3',4,4'-hexachlorobiphenyl. However it has

subsequently been shown that PCB congeners which contain one or no para substituents, are also PB-type inducers (Parkinson et al. 1980b). Some of these compounds include 2,2',3,3',5,5'-hexachlorobiphenyl, 3,3'-dichlorobiphenyl, 2,3,3',5,5',6-hexachlorobiphenyl and 2,3,3',4',5,6-hexachlorobiphenyl. The requirement for at least two ortho chloro substituents conflicts with the reported activity of 4,4'-dichlorobiphenyl and 2,3',4,4'-tetrachlorobiphenyl as PB-type inducers (Yamamoto et al. 1976, Parkinson et al. 1980b). Table 2 illustrates the structurally diverse group of PCBs which are classified as PB-type inducers and it is evident that there are no apparent structure-activity rules for PCBs which enhance this pattern of enzyme activity.

MC-Type Inducers

In 1977, two groups (Poland and Glover 1977, Goldstein et al. 1977) published papers outlining structure-activity rules for PCBs as inducers of cytochrome P-448 dependent monooxygenases (or aryl hydrocarbon hydroxylase, AHH). Their data indicated that PCBs which exhibit this type of induction activity must be substituted in both para positions and at least one meta position on both phenyl rings and not contain any orthochloro substituents. The three compounds defined by these rules, namely 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl are all potent AHH inducers and are approximate isostereomers of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic halogenated aromatic chemical and most potent inducer of microsomal AHH (Poland et al. 1979).

It was apparent that the proposed structure-activity rules did not explain the mixed-type induction activity of the commercial mixtures since the three AHH inducers are only trace components of the active commercial PCBs (Jensen and Sundstrom 1974, Ballschmiter and Zell 1980, Poland et al. 1979). For this reason, the effects of structure on the activity of PCBs as AHH inducers were reevaluated using a series of highly purified synthetic PCB congeners (Parkinson et al. 1980b, Parkinson and Safe 1981, Parkinson et al. 1980a,c, Parkinson et al. 1981a,b, Sawyer and Safe 1982).

Requirements for Para Substituents

The requirements for para substituents were tested using a series of PCB congeners with one, two and zero para substituents and in all cases PCBs which were AHH inducers all contained two para-chloro groups. (Figure 2).

Requirements for Meta Substituents

The requirements for meta substituents were evaluated by testing all the possible meta-substituted PCBs derived from 4,4'-dichlorobiphenyl, a PCB which contains the required two para-chloro groups. Figure 2 denotes the five possible compounds in this series and only the 3,3',4,4'-tetra-, 3,4,4',5-tetra, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls were AHH inducers. Thus one new PCB congener, namely 3,4,4',5-tetrachlorobiphenyl is added to the original list of three AHH inducers and it is apparent that two meta substituents are required for PCBs which induce AHH, however they can be positioned on both or the same phenyl ring.

Table 2. PCBs as PB-Type Inducers

Congener	Number of Chloro Substituent			Reference
	Ortho	Meta	Para	
4,4'-dichlorobiphenyl	0	0	0	Goldstein et al. 1977
2,3,3',4,4-pentachlorobiphenyl	1	1	2	Parkinson et al. 1980b
2,2',4,4'-tetrachlorobiphenyl	2	0	2	Goldstein et al. 1977
3,3'-dichlorobiphenyl	0	2	0	Goldstein et al. 1977
2,3,3',4,5,5'-hexachlorobiphenyl	1	4	1	Parkinson et al. 1981a

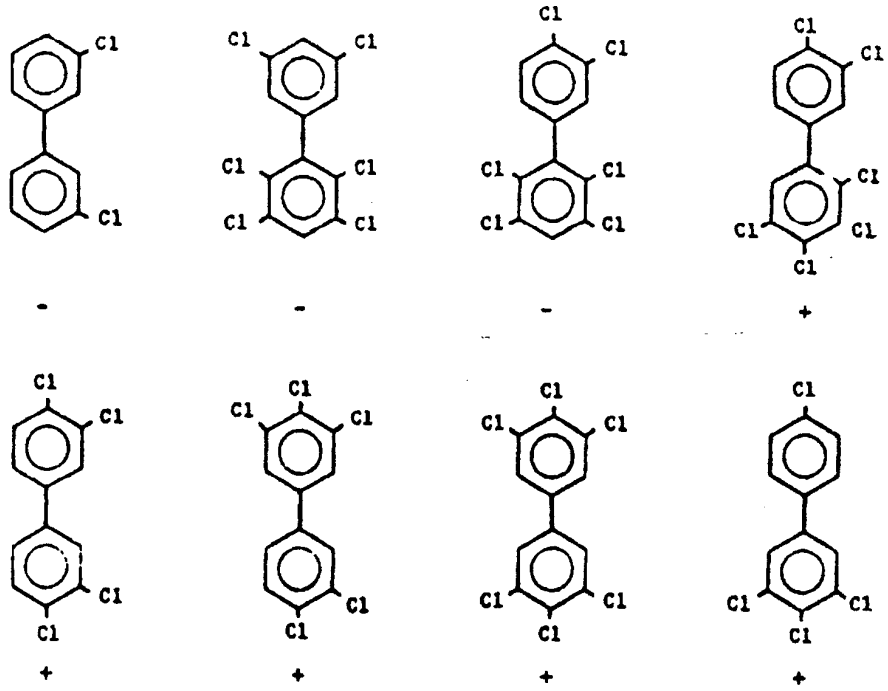


Figure 2. Effects of para and meta substituents on the activity of PCBs as AHH inducers; active (+), inactive (-).

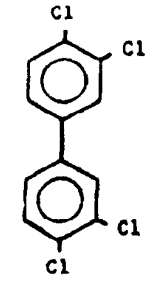
Requirements for Ortho Substituents

A logical approach for evaluating the effects of ortho-chloro substituents on the activity of PCBs as AHH inducers is to synthesize and test all the possible mono- and di-ortho-substituted analogs of the four MC-type inducers. A summary of these compounds is given in Figure 3. All the mono-ortho substituted analogs were mixed-type inducers in the immature male Wistar rat and were inducers of AHH in rat hepatoma H-4-II-E cells in culture. Moreover many of these compounds, including 2,3,3',4,4'-penta-, 2,3',4,4',5-penta-, 2,3,3',4,4',5-hexa- and 2,3,3',4,4',5,5'-heptachlorobiphenyl have been identified as components of the commercial PCBs (Jensen and Sundstrom 1974, Ballschmiter and Zell 1980, Poland et al. 1979).

The effects of two ortho chloro substituents might be expected to have a more pronounced effect on the population of the coplanar conformers and, hence, on their activity as MC-type inducers. Previous reports in the literature suggested that PCBs substituted in at least one meta and para position on each phenyl ring and at two ortho positions were strictly PB-type inducers as evidenced by the activity of the 2,2',3,3',4,4'- and 2,2',4,4',5,5'-hexachlorobiphenyl isomers (Poland and Glover 1977, Goldstein et al. 1977). Contradictory reports have suggested that these isomers also possess MC-type activity (Stonard and Grieg 1976, Alvares 1977). A subsequent reinvestigation of the activity of 2,2',4,4',5,5'-hexachlorobiphenyl as a microsomal enzyme inducer has shown that the MC-type activity of a 99% pure commercial sample (prepared by the Ullmann coupling method) was due to contamination with the highly active 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Goldstein et al. 1978).

In attempt to resolve conflicting reports in the literature, the effects of 2,2',3,3',4,4'-hexa-, 2,2',4,4',5,5'-hexa- and 2,2',3',4,4',5-hexachlorobiphenyl on the hepatic microsomal drug-metabolizing enzymes were evaluated in the immature male rat (Parkinson et al. 1980c). By comparison with the effects of the classical enzyme inducers, PB and MC, 2,2',4,4',5,5'-hexachlorobiphenyl was classified as a pure PB-type inducer. In contrast, 2,2',3,3',4,4'-hexachlorobiphenyl, irrespective of its synthetic route, exhibited PB-type and weak MC-type characteristics; the most prominent feature of the latter being a 7-fold increase in 4-chlorobiphenyl hydroxylase activity. 2,2',3',4,4',5-Hexachlorobiphenyl also resembled a mixed (PB + MC)-type inducer although its MC-type characteristics were more pronounced than those of 2,2',3,3',4,4'-hexachlorobiphenyl. Since these three hexachlorobiphenyls are di-ortho substituted derivatives of the pure MC-type inducer, 3,3',4,4'-tetrachlorobiphenyl, it is clear that the presence of two ortho chlorines does not necessarily abolish MC-type character. A detailed study of all the di-ortho substituted analogs of the four AHH inducers indicated that, in addition to the two PCB isomers noted above, 2,3,4,4',5,6-hexa-, 2,3,3',4,4',6-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl were also mixed-type inducers (Parkinson et al. 1981b).

Therefore the PCBs which induce MC-type activity must be substituted at both para positions, at least two meta positions (but not necessarily on different phenyl rings) and can also contain one ortho substituent; the addition of a second ortho chloro substituent to the nucleus of an MC-type inducer does not necessarily eliminate the qualitative aspects of this activity if one of the ortho substituents is part of a 2,3,4-trichlorophenyl substitution pattern.



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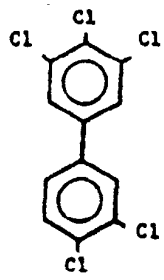

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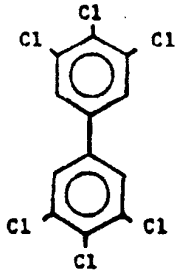

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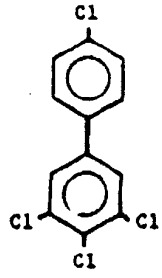

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Figure 3. Mono and di-ortho substituted analogs of 3,3',4,4'-tetra-3,4,4',5-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl.

PCBs Which Do Not Induce Microsomal Monooxygenases

It has also been shown that several PCBs containing one to three chlorine atoms or biphenyls chlorinated on only one of the phenyl rings are weak or inactive as inducer of cytochrome P-450 dependent monooxygenases (Poland and Glover 1977, Goldstein et al. 1977). Their lack of activity may be due to their more rapid rate of metabolism and clearance. Some of the more highly chlorinated octa-decachlorobiphenyls are also inactive (Poland and Glover 1977, Goldstein et al. 1977, Parkinson and Safe 1981). Another group of PCBs which are inactive as AHH inducers contain 3,4 or 3,4,5 substitution on one phenyl ring with a 2,4,6- or 2,3,5-trichloro substitution pattern on the second ring (Parkinson et al. 1981a, Goldstein et al. 1981, Parkinson 1980). These congeners do accumulate in the liver and contain one or two ortho-chloro groups, however, for some reason they are inactive as a microsomal monooxygenase inducer and are relatively nontoxic. The rationale for these results is not apparent.

PCBs: CORRELATION BETWEEN AHH INDUCTION, Ah RECEPTOR BINDING AND TOXICITY

Poland and coworkers have stated "The potency of halogenated aromatic hydrocarbons to induce hepatic AHH activity correlates very closely to their toxic potency" (1980) and "that the toxicity of these chlorinated aromatic hydrocarbons is mediated through the receptor; that is the initial event in the toxic action is the stereo-specific recognition and binding of them by the cytosolic binding species" (Poland et al. 1979). The evidence for this hypothesis is based on the biologic and toxic effects of several congeneric polychlorinated dibenzo-p-dioxins (PCDDs) and halogenated aromatic compounds which are approximate isostereomers of 2,3,7,8-TCDD (Poland et al. 1979). The results which support the proposed correlations are noted below:

1) There is an excellent correlation between the toxic potency of PCDD congeners, their AHH induction activity and affinity for the Ah cytosolic receptor protein (33, for example 2,3,7,8-TCDD is highly toxic, a potent AHH inducer and avidly binds the Ah receptor protein; 2,7,-dichlorodibenzo-p-dioxin is relatively nontoxic, does not bind to the receptor protein or induce microsomal AHH),

2) 2,3,7,8-TCDD and related approximate isostereomers are relatively toxic to the genetically inbred C57BL/6J "responsive mice" and nontoxic to the nonresponsive DBA/2J mice (Poland et al. 1979, Goldstein et al. 1981),

3) 2,3,7,8-TCDD and approximate isostereomers induce AHH in the responsive mice however only 2,3,7,8-TCDD is active (at higher concentrations) in the DBA/2J mice (Poland et al. 1979, Poland and Glover 1980, Poland et al. 1976, Poland et al. 1974).

PCBs are an ideal group of halogenated aromatic chemicals to test and confirm the correlations proposed by Poland and coworkers. Table 3 illustrates the relative AHH inducing activities of 3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl and their mono-ortho chloro analogs. The results illustrate that with few exceptions there is an excellent correlation between the relative potencies of the individual PCB congeners as AHH inducers in Wistar rats and rat hepatoma

Table 3. PCBs - Effects of Structure on Activity

PCB Congeners (number)	Relative Per Cent Activity		
	AHH Induction		
	Cell Cultures	Rats	Receptor Binding
MC-Type Inducers (3)	100-1	+++	100-35
Mixed-Type Inducers (8)	$0.3\text{-}2.4\times10^{-5}$	++	6-1.5
PB-Type Inducers (4)	inactive	inactive	≤ 0.3
Non-Inducers (3)	inactive	inactive	≤ 0.3
2,3,7,8-TCDD	400	+++++	2,500

H-4-II-E cells (Sawyer and Safe 1982) and their avidity for the Ah cytosolic receptor (Bandiera et al. 1982). Although the toxicities of these mono-ortho chlorinated congeners has not been systematically studied there are reports in the literature (Ax and Hansen 1975, Yamamoto et al. 1976, Yoshihara et al. 1979) which confirm the comparatively high toxicity of the 2,3,3',4,4'-penta-, 2,3',4,4',5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl. Current research in my laboratory (Parkinson et al. No date, Safe et al., unpublished results) with genetically inbred mice indicates that administration of the mixed-type PCB inducers to the responsive C57BL/6J mice results in AHH induction and thymic atrophy whereas these compounds do not induce AHH nor are they toxic in the nonresponsive DBA/2J mice.

The results obtained for the PCBs complement the data reported for several PCDD congeners and support the correlations noted by Poland and coworkers (Poland et al. 1979, Parkinson and Safe 1981). Moreover, it is also apparent that PCB congeners which induce AHH activity are toxic and the residue levels of these specific congeners in fish wildlife and exposed humans may be important indicators of potential short and long term toxicity.

PCBs IN HUMANS: PRELIMINARY STUDIES

Figure 3 summarizes all the PCBs expected to exhibit MC-type activity based on the above guidelines and a number of these isomers and congeners have been identified in commercial PCBs and in humans (blood, adipose tissue and breast milk) (Kuwabara et al. 1979, Yakushiji et al. 1978, Yakushiji et al. 1979). As noted in Table 4 there is relatively high concentration of MC-type inducers which preferentially bioconcentrate in human breast milk. A recent study in our laboratory (Parkinson et al. 1980d) has shown that the dose effecting half-maximal (ED_{50}) induction of AHH activity for a reconstituted breast milk PCB sample was ~12 $\mu mol \cdot kg^{-1}$ whereas the ED_{50} for Kanechlor 500, a commercial PCB mixture, was 87 $\mu mol \cdot kg^{-1}$. Thus the increased biological potency of breast milk PCBs reflects the preferential bioconcentration of 2,3',4,4',5--penta-, 2,3,3',4,4'-penta-, 2,3,3',4,4',5-hexa- and 2,2',3',4,4',5-hexachlorobiphenyl which are mixed-type inducers and elicit various toxic responses in the rat, chicken and responsive C57BL/6J mice (Ax and Hansen 1975, Yamamoto et al. 1976, Yoshihara et al. 1979, Parkinson et al. (1982), Safe et al., unpublished results.)

Table 4. Breast Milk and Reconstituted Breast Milk PCBs[a]

PCB Structure	GC Purity (%)	PCB Concentration in Japanese Breast Milk (%)	PCB Concentration in Reconstituted Breast Milk Mixture (%)
2,4,4'-Trichlorobiphenyl	98.5	8.4 ± 2.2	9.2
2,2',5,5'-Tetrachlorobiphenyl	99.0	2.0 ± 1.3	2.2
2,4,4',5-Tetrachlorobiphenyl	99.0	19.1 ± 2.6	20.1
2,2',4,5,5'-Pentachlorobiphenyl	98.5	2.8 ± 0.9	3.0
2,3',4,4',5-Pentachlorobiphenyl	99.0	11.8 ± 1.2	12.8
2,2',3,4',5,5'-Hexachlorobiphenyl	99.0	2.3 ± 0.3	NG[b]
2,3,3',4,4'-Pentachlorobiphenyl	99.0	3.5 ± 0.5	3.9
2,2',4,4',5,5'-Hexachlorobiphenyl	99.0	15.5 ± 0.4	16.3
2,2',3,4,4',5'-Hexachlorobiphenyl	99.0	15.8 ± 0.7	16.9
2,2',3,4',5,5',6-Heptachlorobiphenyl	98.5	3.2 ± 0.6	3.3
2,2',3,4,4',5',6-Heptachlorobiphenyl	99.0	1.6 ± 0.3	1.7
2,3,3',4,4',5-Hexachlorobiphenyl	99.0	2.1 ± 0.7	2.3
2,2',3,4,4',5,5'-Heptachlorobiphenyl	90.0	5.1 ± 0.7	5.7
2,2',3,3',4,4',5-Heptachlorobiphenyl	98.5	2.3 ± 0.3	2.3
Total		95.5	99.9

[a]Kuwabara et al. 1979, Yakushiji et al. 1978, Yakushiji et al. 1979, Parkinson et al. 1980d.
[b]NG = not given.

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DISCUSSION SUMMARY

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The discussion was started by Dr. Kimbrough. She stated that it would not really be possible in the time allotted to comment on all the information presented by Dr. Friess. Because of the Yusho outbreak in Japan and a number of outbreaks of chick edema disease in the United States where the types of compounds that Dr. Safe was talking about had caused illness in chickens because of accidental contamination of food, a number of studies were started with PCBs and related compounds more than a decade ago. It was soon evident that although on a quantitative basis there was a tremendous difference in the toxic effects between PCBs, chlorinated dibenzodioxins, and chlorinated dibenzofurans, that there was a similar response on a qualitative basis that different animal species gave to these compounds.

In humans, chloracne has been reported after exposure to chlorinated naphthalenes, chlorinated biphenyls, the chlorinated dibenzodioxins, and the chlorinated dibenzofurans. This same type of reaction can also be produced in animals, particularly the subhuman primate, but also on the rabbit ear and in nude mice. Before it was possible to detect chlorinated dibenzodioxins chemically, the rabbit ear test was used. Dr. Alan Poland (University of Wisconsin) has now developed a cell culture system where he can produce a keratinizing reaction in the cells. Formation of keratin in the hair follicle is the primary lesion of chloracne, which has nothing to do with juvenile acne.

The other point that was made in the preceding paper was that there have been a number of studies on the carcinogenic effect of PCBs. Some have been negative and others have been positive. If these studies are examined more closely, it becomes obvious why these discrepancies exist. In adequate cancer studies, a sufficient number of animals should be tested over their lifespan. Dogs, for instance, live somewhere between 10 and 14 years. It is therefore not surprising that no cancer was found in a 2-year dog study.

In their bioassay studies, the U. S. National Cancer Institute is now using 50 animals per group. Usually high dosage levels are used because the testing system is not very sensitive. It must first be determined whether there is a carcinogenic effect before extensive studies are done with larger numbers of animals and maybe lower dosage levels. However, dosage levels should not be so high to have the animals lose so much weight that there is a lot of added toxicity. In the study by Kimbrough, et al (1975), only a dietary level of 100 ppm (mg/kg) of Aroclor 1260 was used. Translated into intake by the rats, this means that they in early life consumed a daily dose of 12 mg/kg (because they eat more when they are young). The daily dose dropped later to 5 mg/kg. Only female rats were used in the study. The reason only females were studied was because in an earlier study with only a few rats, one bladder cancer had been noted in a female rat. No bladder

cancer was found in the second study. However, 26 of the rats out of 184 developed hepatocellular carcinomas, and in addition, other pathological changes were noted in the livers, namely, neoplastic nodules also referred to as nodular hyperplasia, and areas of alteration. In the controls, one hepatocellular carcinoma was found in 173 rats and there was little other pathology. None of the liver tumors metastasized.

Because of the confusion about the classification of rat liver tumors, as Dr. Friese mentioned earlier, other pathologists were asked to review this study, such as Dr. Robert Squire, who was director of pathology for the bioassay studies of the National Cancer Institute, and two other pathologists, Dr. Strandberg and Dr. Montali (all authors of the paper). It was realized at the time that some scientists did not consider neoplastic nodules to be tumors. Because of this, a workshop was convened to classify liver lesions (Squire and Levitt 1975). It was the conclusion of the workshop that neoplastic nodules (nodular hyperplasia) were in fact new growths (tumors) rather than something that was just proliferation of tissue. There is still argument about whether neoplastic nodules will in time develop into hepatocellular carcinomas or whether they just occur concurrently with carcinoma.

After the meeting, the National Academy of Sciences decided to go one step further. They convened a group of pathologists chaired by Dr. Harold Stewart to develop a document on the histologic typing of liver tumors of the rat (Institute of Laboratory Animal Resources, 1980).

Another study mentioned as being negative by Dr. Friese was the National Cancer Institute study conducted with the Aroclor 1254 in male and female rats. There were 24 animals per group, which was a somewhat smaller group than the 200 that Dr. Kimbrough used. They gave three different dosage levels, 25, 50, and 100 ppm Aroclor 1254 to the rats over their lifespan. Some rats died early but about 20 or 17 rats per group survived. These rats also developed neoplastic nodules (nodular hyperplasia). At the low dose, neoplastic nodules were seen in 5 males. At the mid dose they were present in 8, and at the high dose they were present in 12. In the females at the low dose, they were present in 6, at the mid dose in 9, and at the high dose in 17. One hepatocellular carcinoma was seen at the mid dose and two at the higher dose in males. In the females, no hepatocellular carcinomas were seen. Comparison of this study with the study of Kimbrough et al. (1975), suggests that if a larger number of rats had been used this study might have been positive.

In the NCI study, a number of exposed rats had adenocarcinoma of the stomach, and this again was not statistically significantly different as was the incidence of the hepatocellular carcinomas. At the low dose and the middle dose, NCI reported one gastrointestinal carcinoma each. Morgan et al. (1981) reexamined a total of 191 stomachs of rats from this study of which 144 rats had been exposed to Aroclor 1254. With different staining techniques, they found six adenocarcinomas in exposed rats, and observed a high incidence of squamous metaplasia in the epithelium of the stomach. Squamous metaplasia is associated with the occurrence of adenocarcinoma in the stomach. The rat strain used was F344. Adenocarcinoma of the stomach in this rat strain is extremely rare, 1 in 1754 (<0.05%). Therefore, they concluded that the like-

lihood of observing 6 adenocarcinomas in 144 rats was less than 0.05 ($P<0.05$). Preston et al. (1981), found that PCBs promoted the induction of cancer initiated by diethyl nitrosamine. This study suggests what was also mentioned by Dr. Safe that PCBs may be promoters. Initiators are thought to cause a primary insult in the cell which in time will cause this cell to become cancerous. Promoters will increase the possibility of an initiated cell to be transformed into a cancerous cell. At the moment there is a lot of uncertainty on how promoters should be regulated.

One problem with the particular compounds we are dealing with here is that these compounds seem to be persistent in the body for extremely long periods of time. Other promoters are not persistent. Perhaps a difference between different groups of compounds based on their metabolic behavior should be made.

Dr. Friess also alluded to the effect of PCBs on the immune response in animals, and Dr. Safe mentioned thymic atrophy and atrophy of the spleen. To bring this into context, there are different types of immune responses. The humoral immune response is consistent with the development of antibodies to common infections and to vaccinations. The cell-mediated immune response, which if impaired, will, for instance, decrease the ability of rejecting foreign tissue grafts. This system is affected when the thymus and spleen are atrophic, and is very sensitive in the early development of children and in young animals.

Reproduction studies were also mentioned and again there seems to be some variation in response for different species. Dr. Friess also pointed out that some animal species are more sensitive to the toxic effects of these compounds than others. If the entire group of compounds Dr. Safe was talking about is examined, it seems that the mink, the guinea pig, the monkey, and the quail or the chick embryo are particularly sensitive while the rat and the mouse are less sensitive. That is also born out in reproduction studies if different mixtures are tested. Dosage levels which produce effects vary widely. There are just a lot of differences in some of the older data that was reviewed by Dr. Friess. Now we are beginning to see why this is so by doing work with isomers which hopefully will eventually put all of this information into place and make it seem more logical.

Dr. Safe recommended that we should perform studies with isomers in animals. For a lifetime study a lot of material is needed. Will these isomers really be available to do these types of studies? Furthermore, Dr. Friess emphasized that some subhuman primates may be more or less susceptible to these compounds than others. Since subhuman primates are very expensive, doing a lot of studies in them would be difficult. Most of the carcinogenesis studies are done in rats and mice because historically we have experience with that system and because rodents have a short lifespan.

Dr. Safe talked about structure activity relationship of the stereoisomers. It seems to be very important whether these chemicals are planar or not. Jim McKinney (NIEHS) is also working in this area. He has recently published a paper in which he points out that co-polarity is also very important (McKinney and Singh 1981). Co-polarity may change in different solutions and with it the amount of chemical that is planar or almost planar can vary. Perhaps Dr. Safe may wish to comment on this.

It was then stated by Dr. Friess that Dr. Safe had a very interesting cellular test system in which the induction of arylhydrocarbon hydroxylase could be measured without using the intact animal system and for the chemicals which induced arylhydrocarbon hydroxylase a dose response curve which went upward with dose could be developed. As the dose was increased even further, the induction did not increase proportionally. Inhibition of excess substrate is a well known biological event in certain enzymes, and Dr. Friess raised the question to Dr. Safe whether if you increase the dose even further, would a point be reached where the induction would decrease to zero? Would an excess of the active isomer, as it were, become its own tool for negating its own effect?

Dr. Friese also raised the question whether in localizing these high isomers in tissues, can a model compound be devised which, in itself, has little biological activity but could block the receptors and block the synthesis points which are triggered by these "hot" isomers. Dr. Safe then responded to these questions. He stated that some isomers are hard to get. They would have to be made. For some, it would be difficult to make large enough quantities for any sort of in vivo studies, but there are a few that are amendable to synthesis.

In response to Dr. Friess' question about less induction at high dosage levels, Dr. Safe stated that this also occurred in vivo with PCBs because the animals are dying. This is probably not due to an inhibition of the enzyme. The cell is killed or the animal is dying. Unfortunately, an overdose would not be protective.

Dr. Mary Wolff then added information about planarity. Dr. McKinney's (NIEHS) thesis is that the achievement of co-planarity is in contradiction to an energy barrier to free rotation around the biphenyl bond. He has shown that there is virtually no rotation. The barrier is low, within 3,4, 3',4'-kinds of materials and goes up when you add ortho halogens but the degree of co-planarity is proportional to the percentage of the time that the two rings remain co-planar. It is important to point out the much greater toxicity of the 2,3,4 and 3,4 substituted isomers but in terms of what is present in people, the Japanese work has suggested that the proportion of PCBs to PCDFs in either Yusho or in their general population people is fairly consistent with what they were exposed to. In breast milk samples, the ratio of PCBs to the PCDFs may then be in the range of one million to one, and of those PCBs by far the larger proportion, certainly greater than 50% are the 2,3,4 substituted kinds of compounds. What does that mean in terms of relative toxicity? Do the 2,3,4 substituted PCBs completely outweigh the potential toxicity of the PCDFs? The chromatograms from breast milk very closely resemble those of the 4,2,4',5' tetrachlorobiphenyl peak on the gas chromatogram. There is also a 2,4,3',4' tetrachlorobiphenyl. That is the only isomer of that series that Dr. Safe failed to mention in his talk.

Dr. Safe reemphasized that polarizability was the point Dr. McKinney was trying to make. He then stated that 2,4, 3',4' is inactive at moderate high doses. The 4,3,4'- is inactive in the chloro series because of lack of polarizability. When one ortho is chlorine added, it is also inactive. Therefore, it makes no contribution. The 2,3,4 substituted biphenyls do predominate in breast milk and in human tissues and some of those analogues are active.

Compared to Aroclor and Kanechlor there is a five-to-sevenfold increase in activity. So, "Yes," they are the ones that can exert toxicity, and there seem to be more of them in human tissue. Humans seem to preferentially concentrate those kinds of chemicals. Jorensenstrom and Jensen confirmed that a long time ago. It is possible that the PCBs which are preferentially retained are also more toxic.

Dr. Muriel Lippman, ERNACO, Inc., added comments to the evaluation of the carcinogenicity data by Dr. Friess. Failure to obtain a statistically significant increase in cancer incidence in treated animals compared to their controls is called a negative study or negative test result. One is tempted to conclude from such a study, or several such studies, that the agent tested is not a carcinogen. This conclusion is not necessarily true. We know that there are many reasons why a carcinogen may yield a negative test result. One such reason is the sensitivity or the power of the experiment to detect the effect.

In order to have a 90% chance of detecting a significant increase in cancer incidence in the treated animals at a P = 5%, which is the customary level adopted, a sample size of 25 animals per group (that is, 25 animals not only in the group but alive at the earliest time to tumor) would require that 9 out of 25 of the animals show the lesion, (in this case the lesion to hepatocellular carcinoma) as compared with 0 out of 25 controls. If the group size is 25 animals per one sex per group, treated and control, there must be a difference of 35% over background in order to be significant at the 5% level to have a 90% chance of detecting this effect.

When the background size is smaller, (e.g., there are 18 animals per group) a higher percentage increase over background is required in order to detect a significant effect. The Calandra study that was discussed by Dr. Friess had at most 14 animals per group. The NCI study had 24 animals per group of such sex or less and the Ito study had 12 in each treated group and only 6 controls. These were therefore extremely insensitive studies. A negative result with such insensitive studies does not permit us to distinguish whether there was no carcinogenic effect or whether the study was simply too insensitive to detect the effect.

In contrast, a positive result on a very insensitive study tells us something about the carcinogenic potential of the compound, that it in fact can be detected at all in such an insensitive study. In the Ito study, there was a 41.6% increase in hepatocellular carcinoma with Kanechlor 500; it is just such an insensitive test. That should carry a significant amount of weight.

Now is there anything that we can do to help to distinguish between the truly negative case or a case of insensitivity? I think there are at least two things that we can do. One is to look at the actual incidence of the hepatocellular carcinoma. Is it very high? Is it, in the experience of pathologists, well above background, and secondly, look at morphological changes that may constitute pre-malignant change. Both of these options are available and can be considered in the overall weight of evidence which I would like to summarize. In the case of hepatocellular carcinoma, there was one very sensitive test in rats, that of Dr. Kimbrough's, where she indicated that she started with 200 animals and in which 14% of the treated animals, or

26 out of 184, showed hepatocellular carcinoma. This was highly significant. There was also a very insensitive test, which showed an increase in mice. This was in mice, the Ito study, with 41.6% incidence. So you have a study in rats and a study in mice that indicated carcinogenicity. Even in the NCI study, a very insensitive study, there was some increase (8%) at the 100 ppm dietary level.

If all of the other data on hepatoma, neoplastic nodules, nodules of hyperplasia and hyperplastic foci are examined, almost all of the experiments showed, especially all those insensitive experiments, not only a significant increase in these lesions, but a dose-related response in these lesions. Not all of the community of pathologists agree that neoplastic nodules and similar lesions are pre-malignant and we would not like to have the whole industry wipe out PCBs on such data; but the fact remains that there are strong data indicating hepatocellular carcinoma with an adequate test as well as supporting positive information with insensitive tests. This shows that the weight of evidence very strongly favors the conclusion that there is a carcinogenic effect. The same thing can be said for the other small studies. Collectively these studies represent a body of evidence that is not as fuzzy as Dr. Friess suggests, but allows us to draw a conclusion until such time as individual isomers which Dr. Safe will be able to provide can be actually tested.

If an Aroclor or a Kanechlor preparation contains 40 to 60 components and only some of these are carcinogenic, then the doses of the mixture are not high at all since the administered dose is not indicated by the administered level but containing all the compounds is only that fraction of the administered dose composed of active agents. Since some of the more toxic components are present only in trace quantities, this would suggest that if they are active at all they must be very potent. The effective dose is not only reduced by being a mixture and active and inactive components; it is also reduced by the factor of differential metabolism and differential pharmacokinetics of these components. So the weight of evidence favors the conclusion that these compounds, these mixtures that have been tested, are indeed carcinogens. The data needs to be refined to determine their potency.

Dr. Friess then discussed several points about the procedures by which animal studies conceived and directed at showing carcinogenicity in animal models then become surrogates for risk assessment in humans and the criteria that are required. There is no questioning the point that an adequately designed experiment is necessary in which the right numbers of animals treated with sufficient numbers of doses followed over a sufficient period of time, approximating a considerable chunk of the lifetime, then followed toward the end of the lifespan to get good dose response numbers for malignancies is terribly important. There is no evading the point that this kind of information should be established for both sexes of animals. Therefore, one would truly want an internationally well-designed experiment with an agreed upon Aroclor, strain, dosing schedule and protocol to follow an internationally approved experiment for the full course of the animal's life, using both sexes of animals. Secondly, if positive results were obtained which were unequivocal, both statistically and biologically, then a similar experiment should be conducted in a second species. If the first was a rodent species, preferably a nonrodent should be used. Again, malignant tumors, not nodules,

not hyperplasia, not metaplasia, not potentially precancerous lesions but malignant tumors should be demonstrated.

What Dr. Friess recited according to him is a kind of international consensus under the umbrella of the International Agency for Research on Cancer in which powerful criteria bearing on the sufficiency of evidence to brand the chemical as a carcinogen in an animal model, and then to use that information for prediction or translation to the human really points to malignant tumors, controlled experiments, large chunks of lifetime and at least two species of animals.

This information to this degree of rigidity does not exist with respect to the PCBs. That doesn't say it shouldn't. The discussion of the meaningful interpretation of potentially precancerous lesions becomes obviated when good experiments are available with or without cancerous tumors. Assuming that two species have shown malignant lesions in a significantly greater incidence over the controls, then the actual exposure patterns of humans should be reviewed, adventitious usually, with occupational patterns as surrogates of the greatest exposures. This is then translated, by use of modeling equations from the incidence rates that you saw in your most sensitive, positive animal model, to the prediction of risk in the human, making the regulatory assumption that the human is taken as being as sensitive as the most sensitive animal model, even if no effects are seen in the human. Having made that risk projection, using a number of empirical projecting equations, you only believe it when the loop is closed and cancer is observed in humans as a function of dose.

There is no denying that you want the perfect experiment, and none of the experiments which were listed as the three best are perfect. Dr. Kimbrough would agree that they are not perfect, and to the tune that they still disagree, and each has flaws and warts, the positive approach is to design and do good, solid, perfect experiments, looking for cancer as the end point.

Dr. Kimbrough then responded that these suggestions are great. The problem is that we don't live in an ideal world and doing these types of studies in two species is very expensive, particularly if non-rodents are used. For instance, a dog study would take at least ten years and subhuman primate studies would last even longer than that. Particularly with the PCBs, an additional question or problem exists which may be quite unsurmountable. As everybody here has stated, there are a lot of differences in the toxicity of the different isomers and congeners. Which mixture should be tested in these very expensive studies? At the Centers for Disease Control, one of our goals now is to prevent disease. Should we take note of any of the animal studies done thus far and try to reduce exposure? Or should we wait until the evidence is there in people, that there actually has been an effect. That is very often our dilemma, and also the dilemma of the regulator. Our foremost goal is to protect the health of the people, and how should that best be done?

We are trying to develop now at the Centers for Disease Control, some ways to evaluate people for earlier indicators of effects of exposure so it will no longer be necessary to wait until death certificates can be sorted.

Dr. Friess stated that he agreed completely. People should be protected on the basis of present reality, and one mode is to reduce all current and future exposure, absolutely.

Dr. Edward Burger of Georgetown University resumed the discussion on structure activity relationships, referring to the elegant work by Dr. Safe which showed some element of success. In Dr. Safe's work, the biological end points that were used to measure toxicity were atrophy of parts of the lymphoid system, the thymus and the spleen. To what extent can this honestly be called a toxic effect, and what does it mean?

Dr. Safe responded that he did not know. Splenic and thymic atrophy were chosen because it can be measured in a short time. When one of your organs shrivel up, if you don't want to call it toxicity, you don't have to. Whereupon Dr. Burger pointed out that the thymus in the human being normally shrivels up with development. It doesn't in other animals, but it does in the human being. If it doesn't, it is an abnormal event, in fact. To understand what atrophy of the thymus in animals may mean he proposed that it may mean that the immune system is to some extent affected in a way that he may not understand because the lymphocytes which are mainstays of the immune system are derived to some extent from the thymus and from other parts of the lymphoid system.

In that case, should indices of disease end points be examined which have nothing to do with cancer or other things talked about in so many of these meetings--namely, immune-related diseases, such as rheumatoid diseases and collagen diseases? Dr. Kimbrough pointed out that unfortunately none of the members of the panel were immunologists. However, in the animal systems at least there is some evidence that if the thymus of the infant mouse is irradiated, then this mouse does not develop properly but develops runt disease. Animals with runt disease may in the end stage of their disease develop leukemia. This was an effect of radiation on the thymus and certain immunosuppressive drugs. In the thirties, the thymus of children was irradiated because at that time the idea existed that a large thymus would somehow cause ill health in children. These children later had a high incidence of cancer of the thyroid because of the radiation exposure, but the other effects that were observed in rodents have not been reported in the children. The types of chlorinated hydrocarbon compounds discussed here, at least in rodents, affect the immune system the way radiation does, but this has so far not been shown in people for most of these compounds.

In order to evaluate the immune system properly in population studies, base lines of normal variations in the general population need to be developed first and laboratory methods need to be standardized.

Dr. Burger then reiterated that he was bothered by the jump that was made by calling the association described as one between structure and toxicity based upon an observation, the meaning of which he does not understand.

Dr. Friess then outlined what he felt were meaningful biological end points for immunocompetence, such as exposure to endotoxins, invading viruses, invading microorganisms of the malarial parasite type, and the more subtle changes in immunocompetence, such as the ability to reject grafts. In summary, meaningful end points are the ability to withstand external challenges of

infectious agents and the ability to respond differently to foreign tissues. Dr. Burger added that there are some disease processes whose bases are thought to be, at least in part, a deranged immune system.

Dr. Zarvins, Westinghouse R&D, then voiced his concerns about the following quote: "Section 761.30, the EPA Administrator hereby finds that any exposure of human beings or the environment to PCBs as measured or detected by any scientifically acceptable analytical method is a significant exposure." Should this be construed to mean that any exposure detected by any analytical method would produce clinically detectable symptoms in humans? Ultimately this whole discussion revolves around what a human response is. In the epidemiological investigations, small that they are, there have been certain responses observed or not observed. Should it be concluded that any level of PCB will produce clinical symptoms?

Dr. Friess in his response indicated that the question about dose versus response could not be resolved from epidemiological studies which were not designed in that sophisticated a fashion, except perhaps for the chloracne effect. Epidemiologists have looked at many exposure situations, over the last 15, 20, 25 years and have concluded that if the folks over long periods of time were exposed to less than 200 $\mu g/m^3$ concentration in air, the chances of developing the signs of chloracne are low. Whereas if the exposure levels for reasonable periods of time, over years, repetitively, were much higher than that, then their chances of developing chloracne are higher. With respect to chloracne, significant exposure really means not the first detectable molecule that entered the body, but that exposure over time which gave a detectable or reasonable incidence of the effect, chloracne.

With respect to the induction of enzymes, no information is available. However, it certainly would be greater than one molecule or greater than the current sophisticated sensitive limits of detection. Another worrisome effect is the information presented on the infants who had low for term weight. Although high and low doses were discussed with the higher dose producing more of an effect, it must be determined whether there is a dose below which the effect goes to zero. Perhaps the effect on birth weight might be linked to enzyme induction, and enzyme induction means the creation of catalysts for oxidation, and hormones and steroids could be oxidized which would effect the growth of the infant in utero. It needs to be determined whether this is a reversible effect. In the case of the Yusho infants, the effect was reversible.

Dr. Kimbrough did not have a definite answer either. However, for many years PCBs have been present in trace amounts in human adipose tissue, in serum, and other tissues. We all carry low body burdens, and we have all ingested trace amounts of PCB. A certain amount of environmental pollution exists that nothing can be done about in the short term. PCBs are not the only thing in the environment. We are exposed to many other things, and to sort this out at the very low trace level is impossible in the general population.

Dr. David Stalling from Fish and Wildlife Service, wanted to know whether Dr. Safe had made any rough comparison between the ortho-unsubstituted PCBs in terms of percent of total Aroclor composition versus percent of total toxicity. He offered an alternative approach reported by him in the Proceedings of the Symposium on PCBs published in the New York Academy of Sciences which offers

an alternative to the large amount of work on pure isomers, namely, that Aroclors can be fractionated on carbon to give elution profiles which are inversely related to the number of chlorines in the ortho positions. Fractions could readily be prepared, which are enriched if not totally containing the 1-chlorine PCB isomers in comparison with the 2,3, and 4 substituted ones to evaluate the toxic response. It must be pointed out that chlorinated dibenzofurans are present in fish, a large element in the human food chain, approximately at a ratio of one part of chlorinated dibenzofuran to 100,000 parts of PCBs.

In the published literature on chlorinated dibenzofurans and PCBs, it is safe to say that most of the chlorinated dibenzofurans in Aroclors are under 10 ppm, probably closer to 1 ppm. Dr. Rappe's group (Umea, Sweden) has done quite a bit of work on this, but there are about 35 isomers of chlorinated dibenzofurans in PCBs. In the environment about four or five isomers are found, all of which appear to be 2,3,7,8 substituted, and with the appropriate calculations, it can be determined that a 100-fold excess of 2,3,7,8 substituted chlorinated dibenzofurans exists in the fish that we have analyzed. This raises a lot of questions, particularly since the findings made in fish residues points to the 2,3,7,8-substituted dibenzofurans, exactly analogous to the residues in Yusho oil.

Dr. Safe responded that his group had experiments in progress to determine how much this "kind of new class" of toxic PCBs contribute, but no results are available. Nor is it known what the contribution of the polychlorinated dibenzofurans to the overall toxicity of PCBs is. However, the point must be made that the PCBs can contribute. They are toxic, and what proportion of this is either in fish or in Yusho is not known, but they are contributing and not everything can be blamed on polychlorinated dibenzofurans.

Dr. Edward Feeder, Southern California Edison Company, pointed out that Dr. Blair Smith from the National Institute of Occupational Safety and Health had only found equivocal changes in mortality patterns and no clear association with carcinogenic effects in his studies of workers exposed to PCBs in capacitor plants. These workers had PCB blood levels as high as 500 ppb (ug/l). Perhaps blood PCB levels achieved in the animal studies could be compared to these extremely high human levels and then conclusions could be drawn relevant to dose effect levels and responses or lack thereof in humans?

Dr. Friess in his response focused on the three key animal studies which went long enough and pointed out that this information was not available. In the human epidemiological studies that were reviewed at the meeting, exposure levels and some blood levels are available but with no clear indicators of excess carcinogenesis we might have essentially the negative information but we don't have the threshold for the positive information.

Dr. Kimbrough added that she did not analyse the tissues for PCBs in her animal study and neither did anybody else in any of the other studies. However, it is very important to actually find out what the tissue levels are that cause an effect in target organs. An additional problem is differences in species response where some species may be more susceptible at lower dosage levels.

Whereupon Dr. Faeder responded that that information could be developed from general single species pharmacokinetic studies. Is there any data for other effects which make these comparisons and determine blood levels so that in fact, in looking for similar situations in humans interspecies comparisons could be made?

Dr. Friess then pointed out that the only example was the chloracne epidemiological information where 200 $\mu g/m^3$ in air was associated with a higher incidence of chloracne. This probably goes along with a blood level of about half the air level. Perhaps 100 ppb in blood or higher may be associated with a higher probability of developing chloracne, but it sure "as heck" hasn't been proved as causation.

Dr. John Brown of General Electric Research and Development Center, emphasized that he was quite concerned about the interpretation of chronic health effects observed in animals that may or may not be chronically accumulating the agent being tested because in order to interpret such data intelligently and make interspecies comparisons, one would like to know the level of the agent present at the time the health effects are being observed. In Dr. Kimbrough's first chart where she described the feeding schedule to the rats that were administered Aroclor 1260, it was illustrated that those rats received 3 or 4 g/kg of Aroclor 1260 as the total dose. Matthews has reported that Aroclor 1260 is almost entirely retained in the rat which would imply that those rats were carrying on the order of 3 g/kg of Aroclor 1260 at the time of sacrifice, which is approximately the LD_{50}. It is hardly a trivial load. Is there any evidence of tissue levels present in the carcinogenicity tests that have been done to date or has anyone retained tissue so that it may be possible to go back and figure out whether tolerance levels were exceeded in the testing.

Dr. Kimbrough responded that she would have to see whether she still had any tissues. In some studies where PCBs were fed for a period of time and then the rats were removed from exposure at the end of the study, the levels of PCBs were still very high in adipose tissue, as high as 1000 ppm, but since the study was different, such extrapolations should perhaps not be made. The adipose tissue levels might have been higher, and of course the amount that would be in the liver would be proportionally lower. With polybrominated biphenyls this was recently tested because it is very important. The problem always is that the person that does the cancer research works by himself or herself and the chemist does the same thing; and the two people never get together which is very unfortunate. Things should be done differently.

Dr. Kenneth Chase of Washington Occupational Health Associates then expanded on clinical symptoms observed in humans. Traditionally in clinical medicine, we distinguish between symptoms and metabolic abnormalities that are not necessarily accompanied by symptoms and certainly there are a number of studies, many of which have been discussed here, which have documented metabolic abnormalities. For symptoms resulting from PCB exposure, it would be useful to distinguish between acute, subacute, and chronic exposure. When workers are exposed to PCB containing fluids, particularly in heated form, they can get irritation of their skin, their eyes, the mucous membranes with complaints of throat irritation and a cough. Those symptoms go away when the worker is removed from exposure. Not every single worker reports those symptoms, but

they are very consistent. Nobody makes much issue over those symptoms, but as long as that question was being raised, it should be pointed out that there are acute symptoms associated with acute exposure to PCBs. Chloracne and other dermatitis may be associated with acute and longer exposure. Dr. Friess made the observation that there seems to be a threshold at which chloracne is observed in terms of work environment exposure. The figure of 200 $\mu g/m^3$ was suggested. There are quotes like that also in the NIOSH criteria document, etc. In our experience, most of the PCB exposure is dermal and not inhalation, although that myth keeps getting perpetuated. PCBs as the chemists can tell us don't have a high vapor pressure, but they are easily absorbed through the skin. There are studies that were cited yesterday by Dr. Gaffey that suggest that the likelihood of developing chloracne probably has an awful lot to do with work practices and work habits, and those statements are elaborated on in our paper, in Smith's paper, and also in Maroni's paper (Maroni et al. 1980), probably the only investigator who tried to do measurements of PCBs on the skin itself.

Dr. Mary Jo Vodicnik, Medical College of Wisconsin, then commented on the reproductive effects ascribed to the PCBs in light of Dr. Friess' statement that induction of the P450 system may result in an increased metabolism of sex steroids, and could be related to the lower birth weights. It has been suggested that some of the reproductive effects which have been noted in both rodents and subhuman primates, as well as menstrual irregularities, are due to an increase in the metabolism of sex steroids. While it is important to examine reproductive effects in sexually mature animals, it is going to be very important to consider these effects in the neonate.

It has been shown that offspring of mothers in rodents pretreated with polybrominated biphenyls are reproductively incompetent later in life. It has also been shown that circulating sex steroids in the neonate are responsible for normal reproductive development, and it is probably via the mechanism of these sex steroids acting on the hypothalamus to imprint it during the neonatal period. This is probably also true of the sex difference in the hepatic mono-oxygenase system seen in sexually mature animals. Since PCBs freely enter breast milk and since many of the congeners found in breast milk are potent inducers, the effects of PCBs on the monooxygenase system in the neonatal period should be investigated more closely.

Dr. Blair Smith of NIOSH pointed out, having witnessed knowledgeable dermatologists argue over whether or not particular lesions are chloracne, that the diagnosis is largely dependent upon the clinician's pre-existing knowledge of exposure. This suggests that the diagnosis of chloracne is not as hard a sign of PCB exposure or toxicity as we might like to think. There is a lot of inter- and intra-observer variability in the diagnosis. The number of cases actually cited are sufficiently small to raise the question whether these observations are not in some way biased by the investigator's knowledge of the exposures.

The other biological outcome which has been cited as a hard or harder biological effect that can be observed in humans, are the birth weights of infants born to PCB exposed mothers, the only human data from the U.S. was in Chapter 5. It was a comparison of PCB high exposed, low exposed, and not exposed (control) women in New York State. The results compared to the

control group were inconsistent. The mean birth weights were lower for the high exposed than for the controls, and they were higher for the low PCB exposed group than for the controls. The confidence intervals cited by the investigator were 90% confidence intervals. In effect, one has three comparisons at a P value that is really 10% in looking at that data, and the results as presented could have been due merely to chance.

Dr. Kimbrough ended the discussion by agreeing that as far as the comment on chloracne is concerned, clinical observation are an imprecise science. Physicians that have not had a lot of experience with chloracne might not even detect mild cases of chloracne. On the other hand, other lesions are misclassified as chloracne. However, if a biopsy is taken of a hair follicle, it should be possible to diagnose the lesion microscopically. As far as the birth weights are concerned, there is evidence in animals that reproduction is effected. For that reason, and the preliminary results in humans presented here, this problem should be explored further. A lot of other things effect birth weight, such as smoking and alcohol consumption by the mother. In the human population, it will be difficult to sort these things out. Confounding variables are one of the biggest problems in these types of human studies.

Addendum

In an additional study with Sprague Dawley rats fed 100 ppm (mg/kg) Aroclor 1260, in which there were 32 male controls, 49 female controls, 46 male exposed, and 47 female exposed rats available for microscopic examination, a high incidence of well differentiated hepatocellular carcinomas were found in female rats and a lower incidence in males (Weltman, R.H. and Norback, D.H., 1981).

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CHAPTER 7

ENVIRONMENTAL EFFECTS

The paper in this chapter describes several recent studies of the environmental effects of PCBs. Pre-1978 papers are recognized, but the trend toward fewer acute and more chronic studies, and better quality reports is apparent. The author discusses PCBs distribution within the aquatic environment, effects on phytoplankton production, distribution among different fish trophic levels (e.g., planktivores, detritivores, and carnivores), and the use of more sensitive 'indicator' species to monitor overall PCBs levels and locate "hot spots". Also included is the effects of current PCBs levels on sensitive nonaquatic species, such as reproduction and lethality in the mink, or heritable metabolic effects in fish-eating birds. Hypothesized mechanisms for the partitioning of PCBs in water and lipid material are discussed, as are the problems associated with the absence of a standard data collection and reporting format for these types of studies.

The discussion summary begins with a presentation demonstrating the relationship between very low levels of PCBs contamination and measurable increases in fish liver monooxygenase activities. This describes the rate of PCBs bioaccumulation and enzyme induction in these species, and the disappearance of the effects following removal of the contaminant, suggesting a possible model for monitoring the time course of PCBs contamination. The question is raised as to how reasonable or appropriate the use of monooxygenase activities might be as an indicator of toxicity. Also discussed is a comparison of PCBs levels which induce enzyme and reproductive effects versus frank toxicity, and the comparative toxic levels of dibenzofurans in fish.

ENVIRONMENTAL EFFECTS OF POLYCHLORINATED BIPHENYLS: IMPORTANT ECOLOGICAL ASPECTS

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The past few years have been marked by a prismatic growth in studies of the environmental effects of polychlorinated biphenyl (PCB). Although studies may not have increased on a quantitative basis, the qualitative growth of such research efforts has been impressive. Reports of PCB residue levels in limited populations from small areas have been replaced by studies of several components of the ecosystem over a large geographical reach. Attempts are now being made to construct sophisticated models to predict residues in various environmental components based on input of PCB to the system. Acute toxicity studies have given way in many cases to studies of chronic toxicity which propose the mechanism producing the observed effect. Many of the advances in the toxicological techniques which are a part of this prismatic growth have been detailed by Mayer et al. (1980).

The purpose of this paper is to review recent (generally post-1978) literature concerning the effects of PCB on the environment. Original data concerning the PCB content of selected freshwater fish species is presented as an example of areas requiring additional investigation. The implications of these findings for establishing water quality standards and designing more efficient PCB monitoring programs are discussed briefly.

RECENT ECOTOXICITY STUDIES

There is little doubt even relatively modest levels of PCB can adversely affect the very base of the aquatic trophic pyramid. Aroclor* 1248 has been shown to significantly depress the growth of a green algae at aqueous concentrations of 11 to 111 µg/l (Christensen and Zielski 1980) and 10 µg/l PCB inhibited growth of phytoplankton at a variety of cell densities (Kleppel and McLaughlin 1980). In the latter study, a recovery of growth occurred after the initial inhibition and this was believed to be partially dependent on population size and physiological status at the time of exposure. Only 1 µg/l of Aroclor 1254 reduced the biomass and size of phytoplankton communities (O'Connors 1978) and reduced cell division of the alga Isochrysis galbana (Harding and Phillips 1978). Fifty µg/l of PCB significantly reduced the uptake of ^{14}C by the marine diatom Thalassiosira pseudonana through reduced photosynthesis per cell and a decrease in viable cells (Michaels et al. 1982). Reduced growth also occurred in the alga Euglena gracilis upon exposure to 10,000 µg/l of Aroclor 1242 for eight days (Bryan and Olafson 1978). A yeast exposed to several species of Aroclor at concentrations of 25,000 µg/l displayed reduced growth after 160 hr (Tejedor 1978). In addition to decreased growth, other effects

*Aroclor is a registered trademark of the Monsanto Chemical Company, Incorporated.

of PCBs have been observed. An alga exposed to 50 µg/l of PCB for only three hr showed reduced motility (Zullei and Benecke 1978). Exposure of the green alga Dunnaliella tertiolecta to 100 µg/l of Aroclor 1254 resulted in increased cell division (Harding and Phillips 1978), a phenomenon similar to the action of some herbicides.

The fact various species of plankton within a population may be affected at different levels of PCB contamination has important ramifications for aquatic communities. Those organisms most sensitive to PCB will be eliminated from the population at relatively low levels of PCB input. This creates voids which are filled by less PCB sensitive species, thus causing a shift in species composition of the population. The resulting alterations to aquatic food webs are almost incomprehensible and may adversely affect organisms otherwise quite tolerant of PCB contamination.

Current studies have attributed chronic effects noted for other organochlorines to PCB (Mehrle et al. 1982). These authors observed a decrease in the mechanical properties of vertebrae of striped bass (Morone saxatilis) collected from the Eastern U.S. They believe this decrease to be a result of environmental contaminants, the most common of which was PCB. Whole body content of PCB residues in these striped bass ranged from 0.03 to 2.6 µg/g (wet weight). The mechanism involved was related to an induced functional deficiency of Vitamin C, leading to a reduction in collagen formation, with the increased ratio of minerals to collagen causing brittleness of the spine. This hypothesis was originally advanced for toxaphene (Mayer et al. 1978, Hamilton et al. 1981) and similar effects have been shown to result from kepone and mirex (Mehrle et al. 1981). The identification of this process represents a new direction in PCB ecotoxicology research. Not only does it illustrate chronic processes perhaps common to a variety of toxic substances, it presents pathways for research into countering these effects.

The more traditional investigations focusing on bioassay tests are less well represented in recent studies than in the past. Perhaps this indicates a shift of emphasis to the effects of toxic substances other than PCB or it may be symptomatic of a general decline in research funding. Acute toxicity of PCB to rainbow trout (Salmo gairdneri) in flow-through tests was measured as 2 µg/l (Birge 1979). These same investigators reported LC50s for largemouth bass (Micropterus salmoides) and redear sunfish (Lepomis microlophus) of 2.3 µg/l and 19 µg/l respectively. Chronic effects on the life cycle of brook trout (Salvelinus fontinalis) occurred at concentrations of Aroclor 1254 ranging from 0.7 to 1.5 µg/l (Mauck 1978). Fathead minnows (Pimephales promelas) exhibited chronic effects at concentrations of 0.1 to 0.4 µg/l of Aroclor 1248 and 1.3 to 4.0 µg/l of Aroclor 1260 (DeFoe 1978). The 30-day LC50 obtained for fathead minnows exposed to Aroclor 1248 was 4.7 µg/l, the value for Aroclor 1260 was 3.3 µg/l (DeFoe 1978).

Bioassays conducted on shrimp (Crangon septemspinosa) produced a 96-hour LC50 for Aroclor 1242 in seawater (20° C) of 13.0 µg/l while the same test using Aroclor 1254 resulted in an LC50 of 12.0 µg/l (McLeese and Metcalfe 1980). Threshold levels were 6.5 and 0.5 µg/l respectively. An attempt to determine the 96-hour LC50 for the two PCB species in sediments resulted in no mortality of the shrimp at 780 µg/l of Aroclor 1242 and 3400 µg/l of Aroclor 1254 (McLeese and Metcalfe 1980). Gulf killifish (Fundulus grandis) exposed

for 24 hr to water containing 40 µg/l Aroclor 1242 had highly significant ($p=0.001$) increases in locomotor activity (Fingerman and Russell 1980). Aqueous concentrations of 8 µg/l Aroclor 1242 inhibited molting in fiddler crabs (Uca pugilator) exposed for 38 days (Fingerman and Fingerman 1979) while crabs exposed to 2000 µg/l Aroclor 1242 for four days exhibited a greater dispersion of melanin in their carapaces (Fingerman and Fingerman 1978).

The information obtained from these recent studies on the ecotoxicity of PCB reinforce the wealth of information published during the early 1970s. Invertebrates, as a group, are among the organisms most sensitive to PCB, and salmon and trout are more readily affected than other fish species. In general, toxicity appears to increase with the level of chlorination in a particular PCB species. Data for chronic toxicity studies on many common freshwater fish species is lacking and there is no bioassay information for entire groups of organisms. This is particularly true for wild mammals. Animals which are large, difficult to keep under laboratory conditions, or rare are not represented in the literature on PCB toxicity. This may be especially important since rarity or difficulty in surviving captivity might be indicators of hypersensitivity to environmental perturbations. An early caution by Johnson (1968) is applicable to data on the highly lipid-soluble PCBs. He noted laboratory diets artificially high in fat might cause an apparent increase in body content of organochlorines. Similarly, an improper diet might cause utilization of body fat containing PCB, thus increasing apparent toxicity. Methods of reporting the results of chronic toxicity studies of PCB are also subject to improvement. A minority of the investigators calculated effective concentrations (EC) for a given affect, although this is an excellent technique. Likewise, few studies presented threshold concentrations for the test organisms.

IMPACT OF PCB ON REPRODUCTION

Fish

Disruption of reproductive processes in fish exposed to PCB apparently is not related to decreases in hatchability of eggs. A comparison of eggs from wild populations of Lake Michigan lake trout (Salvelinus namaycush) with eggs from hatchery stock found no effect of PCBs on the early survival of the trout (Stauffer 1979). In that study, the eggs from the wild populations contained PCB residues of 3.16 to 9.9 µg/g, those from the hatchery fish ranged from 0.20 to 0.28 µg/g. Studies of Atlantic salmon (Salmo salar) also showed no correlation between PCB levels in eggs and hatchability. The salmon eggs contained 1.88 to 6.48 µg/g PCB on a lipid basis (Zitko and Saunders 1979). PCB also had no effect on the hatchability of dried brine shrimp (Artemia salina) eggs when hatching occurred in water containing concentrations of 10 mg/l PCB (Kuwabara et al. 1980).

Birds

There are few reports of PCB adversely affecting reproduction in birds. Japanese quail (Coturnix coturnix japonica) fed diets containing 50, 100, and 150 µg/g PCB for 3 weeks during sexual maturity showed significant effects. These included delayed laying and diminished laying capacity, increased liver weight, testes changes, and reduced breaking strength of eggs (Biesemann

1982). Experimental evidence obtained for Western grebes (Aechmophorus occidentalis) indicates PCB levels are not directly correlated with eggshell thinning although an interaction between Aroclor 1260 and DDE in reducing eggshell thickness was suggested (Lindvall and Low 1980). Screech owls (Otus asio) fed a diet containing 3 µg/g of Aroclor 1248 showed no decrease in eggshell thickness, number of eggs laid, young hatched, or young fledged (McLane and Hughes 1980). The eggs of these owls contained PCB residues ranging from 3.9 to 17.8 µg/g. There was no evidence of eggshell thinning in Great skua (Catheracta skua) collected from several locations. The average PCB content of the adult skua muscle tissue was 16.0 µg/g (wet weight); the eggs averaged 20.0 µg/g (wet weight) (Furness and Hutton 1979).

A survey of six bird species; laughing gull (Larus atricilla), white ibis (Eudocimus albus), glossy ibis (Plegadis falcinellus), American oystercatcher (Haematopus pallistus), willet (Catoptrophorus semipalmatus), and ruddy turnstone (Arenaria interpres), revealed no consistent trend of PCB in the eggs (Blus and Lamont 1978). These investigators found no obvious problems with reproductive success in any of the species. In a similar study conducted in Canada, the mean PCB content of eggs ranged from 0.52 µg/g in common eider (Somateria mollissima) to 21.7 µg/g in razorbill (Alca torda) (Pearce et al. 1979). Those authors also concluded those species were unaffected by PCB levels. Nine-month old mallard ducks (Anas platyrhychus) fed a diet that included 25 µg/g of Aroclor 1254 for at least one month displayed no ill effects on reproduction. The parameters measured included the number of hens laying, the date of the first egg, clutch size, hatching success, survival to three weeks, number of times off the nest per day, and total time off the nest (Custer and Heinz 1980). There was a large increase in fertility in the male ducks fed the PCB diet which the authors were unable to explain.

The effect of PCB on the reproduction of birds may be more insidious than indicated in the previous review. Chickens (Gelus gelus) receiving 5 µg/g of PCB in their diet showed no significant effects on reproduction. However, female progeny of these chickens displayed significant decreases in the biological effectiveness of estradiol as well as other alterations to their metabolism (Kosutzky et al. 1979). Significant decreases in body temperature at ambient temperatures of 0 and -5° C occurred in mourning doves (Zenaidura macroura) fed a diet with 40 µg/g Aroclor 1254 for 42 days (Tori and Mayer 1981). The investigators concluded PCBs could reduce winter survival of adult and juvenile mourning doves. This lack of information on the effects of PCB on progeny of birds represents a needed area of research and reemphasizes the well-known need for studies measuring the effects of toxic substances on several generations. It is probable there are populations of piscivorous birds in the U.S. receiving 5 µg/g of PCB in their diet.

Mammals

Little information is available concerning the effects of PCB on wild mammals although some information exists on residue levels in natural populations. Wild populations of big brown bats (Eptesicus fuscus) were found to contain elevated levels of Aroclor 1260 and to have high stillbirth rates, but experiments failed to correlate the two (Clark 1978). Similar studies on little brown bats (Myotis lucifugus) indicated high levels of PCB (up to 25

µg/g) in the fetuses may have been responsible for some stillbirths (Clark and Krynitsky 1978).

Mink (Mustela vison) fed diets containing Aroclor 1242 experienced total reproductive failure when PCB levels in their feed reached 5 µg/g and all mink died when the diet contained 20 µg/g of PCB. Ferrets fed the same levels of PCB showed no adverse effects of 5 µg/g but were unable to successfully reproduce at dietary levels of 20 µg/g PCB (Bleavins et al. 1980). Earlier literature identified mink as being especially sensitive to PCB and it has been estimated 65% of the fish samples collected in 1978 from major watersheds near the Great Lakes would pose significant hazards to animals, such as mink, feeding on the fish (Veith et al. 1981).

MODES OF PCB ABSORPTION

Conflicting information is available on the mechanisms by which the biota may become contaminated by PCB and this is certainly a major area requiring further study. Bjerk and Brevik (1980) provide three mechanisms by which PCB may contaminate an animal: 1) across the skin, 2) through respiration, and 3) ingestion. In aquatic investigations, it is difficult to separate PCB obtained from water and that received through respiration, so many investigators have combined the categories. Such a mechanism can act rapidly; larval striped bass exposed to aqueous concentrations of Aroclor 1254 obtained 80% of their final PCB concentration in the first 12 hr (Califano et al. 1980). Brevik (1981) noted PCB content was not age-dependent in several fish species from a Norwegian lake. He believed this was evidence of PCB partitioning between fish tissue lipids and surrounding water and stated this mechanism was the predominant factor in determining PCB levels in fish. A 5-year monitoring study of fish in a Finnish lake also revealed PCB concentrations in fish were correlated with fat content (Hattula et al 1978). The same findings of a correlation between lipid levels and PCB content was reported by van den Broek (1979). Significant correlations between PCB and lipid content have been found for yellow perch (Perca flavescens) and salmonids in Lake Ontario (Armstrong and Sloan 1980). Indeed, there appears to be a constant partitioning of PCB between the various components of the environment. Partitioning of PCB between the water and particulate matter has been demonstrated (Biggs et al. 1980). At ^{14}C-PCB concentrations of 25 mg/l, 19 to 22% of the PCB was sorbed to particles and 70 to 72% was in the water. When PCB concentrations were increased four-fold, 66 to 69% was sorbed to particles and 22 to 23% was in the water. This sorption was shown to be reversible.

Unfortunately, the concentration of PCB in an organism is not simply related to the lipid content of a particular tissue. One investigator found no increase in PCB levels in liver tissues of exposed fish, although he was able to correlate sample lipid content and PCB level (van den Broek 1979). He viewed this as evidence of food chain accumulation. PCB content in grey mullets (Chelon labrosus) was not dependent on age but was dependent on food intake (Narbonne 1979). A study by the State of New York found no significant correlation between PCB concentration and lipid content in striped bass (New York Dept. Environ. Conserv. Report 1979) and Mehrle et al. (1982) also found no significant correlation between PCB content and lipids in striped bass. Studies by Hunter et al. (1980a,b) correlated PCB content with feeding habits in several freshwater fish species. Levels of PCB in seal brains were lower

than other tissue of a similar fat content, suggesting differential absorption of PCBs in fat (Rosavell et al. 1979).

No matter what the mechanism, animals can concentrate extremely large amounts of PCB from the environment. In 14-day live car studies in the Hudson River, fish concentrated PCB to levels 15,000 to 26,000 times those in the water (Skea et al. 1979). The importance of deposition of PCB absorbed to suspended particles has not been overlooked. Two species of marine benthos were able to concentrate PCB from 3.8 to 10.8 times the amount in the sediment in 32 days (McLeese et al. 1980). Two species of polychaetes had 5-day bioconcentration factors of 236 to 373 (Courtney et al. 1978), and bioconcentration factors of PCB in fillets of 5 fish species to PCB in sediments ranged from 0.319 to 10.319 (Hunter et al. 1980b).

ECOLOGICAL PROCESSES IN PCB CONTAMINATION

There are two aspects of PCB contamination which have become especially important in the last few years. They are the use of indicator organisms and the ecological processes related to accumulation of PCB in the biota.

Data recently collected from nine Oklahoma lakes illustrates the importance of both areas of PCB investigation. All of the lakes examined are Corps of Engineers impoundments located in the eastern one-half of Oklahoma (Figure 1). The lakes vary greatly in morphometric factors, water quality, age, and watershed industrialization (Table 1). Seven of the lakes have not been identified as having PCB sources in the immediate watershed. Relatively severe PCB contamination was detected in the biota of Ft. Gibson Lake in 1979. An extensive investigation by an inter-agency task force led to the discovery of several small, generally low-level sources of PCB within an industrialized area at the upper end of the lake (State of Oklahoma Report 1980). Webbers Falls Lake has an interim-NPDES permitted PCB discharge into the upper lake. A paper recycling operation has discharged effluent containing a maximum of 2.5 µg/l PCB since 1976.

Methodology

Three-hundred and seven fish representing seven common species were collected from the nine lakes between 1979 and 1981. All fish were collected during the summer months with the aid of gill nets and electro-fishing devices. Sample numbers were determined based on the surface area of the lake, with approximately one fish per 350 to 450 surface ha of water at normal pool elevation. An exception to this rule occurred at Lake Eufaula, where due to budgetary constraints, only one-half the required fish were collected. The samples were primarily composed of about 65% detritivores, 25% carnivores, and 10% planktivores. In some cases it was not possible to collect all seven species. To examine the influence of fish age on PCB burden, desired size ranges were established for each species (Table 2). These were based on length-age relationships (Lewis et al. 1968) and were typically 2-,3-, and 4-year old fish.

Fish species were grouped into three trophic levels as follows:

Planktivores: Gizzard shad (Dorosoma cepidianum)

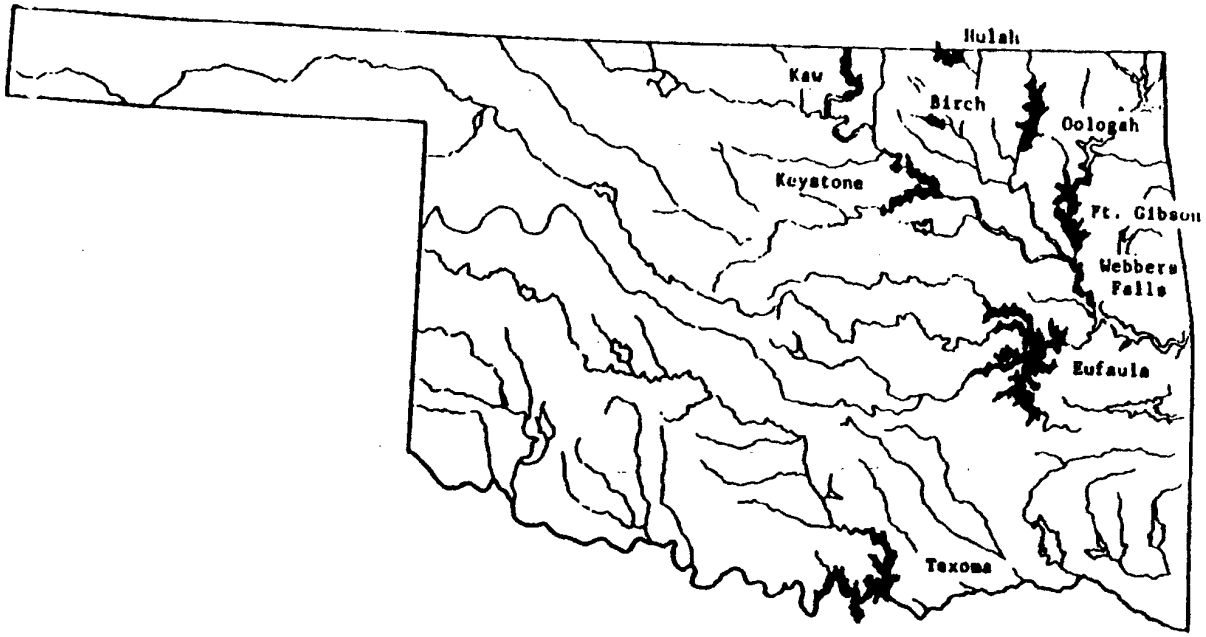

Figure 1. Map of the study area showing lakes sampled.

Table 1. Selected Morphometric Data for Nine Oklahoma Lakes

Factor	Impoundment								
	Eufaula	Oologah	Ft. Gibson	Texoma	Webbers Falls	Birch	Hulah	Keystone	Kaw
Age (years)	17	17	31	37	11	5	30	17	6
Surface Area (ha)	41,279	11,922	7,730	35,613	4,411	1,516	5,261	22,388	15,379
Mean Depth (m)	6.9	5.7	5.8	9.1	5.0	9.9	7.4	11.0	11.8
Maximum Depth (m)	24.7	18.3	21.9	35.4	13.3	20.3	14.0	27.6	21.7
Morphoedaphic Index	10.5	11.7	11.4	13.3	36.3	6.2	9.4	60.2	42.3
Storage Ratio	0.50	0.31	0.07	0.75	0.20	0.34	0.33	0.60	0.52
Mean PCB in water (µg/l)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Table 2. Size Ranges of Fish Collected from Nine Oklahoma Lakes

Species	Minimum size (mm)	Maximum size (mm)
River carpsucker	200	350
Carp	200	350
Channel catfish	200	350
Smallmouth buffalo	200	350
White bass	200	350
Gizzard shad,	125	300
White crappie	150	300

Detritivores: Carp (Cyprinus carpio), channel catfish (Ictalurus punctatus), smallmouth buffalo (Ictiobus bubalus), river carpsucker (Carpiodes carpio)

Carnivores: White crappie (Pomoxis annularis), white bass (Morone chrysops).

Although the feeding habits of some of these species overlap, these groupings provide general trophic levels. The term detritivore may be criticized as too general, however the important point is fish in this group inhabit the bottom substrate. The species in this study were chosen not only based on trophic level, but also because, with the exception of gizzard shad, they are prime contributors to the commercial or sport fishery. It should also be noted the species are common throughout the study-area and are easily collected.

A fillet was removed from each fish as prescribed by the Food and Drug Administration (USFDA 1972), wrapped in aluminum foil, and frozen. The fillets were then transported to a laboratory for determination of total PCB residues. Laboratory analysis followed methods recommended by the FDA (1972). The samples were analyzed using a gas chromatograph with a ^{63}Ni electron-capture detector. Due to contractural constraints, it was necessary to utilize four laboratories for sample analysis. For this reason, particularly strict quality control requirements were maintained. These included sequential recovery and spiked recovery analysis for 5% of all samples. Sequential recovery yielded a minimum of 96% recovery from initial extractions while spiked recovery ranged from 89 to 108%, and the results presented in this study were not corrected for recovery. Replicate analyses were conducted for 5% of all samples and were within 9% of the sample mean. Reagent/glassware blanks were required with each 20 samples. The minimum detection limit for total PCBs in fish flesh was 30 µg/kg, and all results were expressed on a wet-weight basis.

Statistical analyses of the data were conducted with the Statistical Applications System (SAS 1982). The analysis of variance function of the GLM procedure was used to compare PCB residues in fillets to fish species, trophic level, lake, fish length (within a species), and sample location (within selected lakes). When a significant difference ($p=0.05$) was indicated by the analysis of variance, Duncan's multiple range test was used to locate the variance ($p=0.05$). Samples found to contain nondetectable levels of PCB were assumed to contain 15 µg/kg for statistical purposes.

Results

Total PCB content in fish from the nine lakes ranged from not detectable to 16,100 µg/kg and varied significantly ($p=0.0001$) between lakes. Two hundred forty-one (78.5%) of the fillets contained detectable levels of PCB residues and the mean concentration for all samples was 631 µg/kg. The greatest mean concentrations occurred in fish from the two lakes with known PCB sources; Ft. Gibson and Webbers Falls. Each of these lakes exhibited significant variation in PCB residues in fish while the remaining lakes comprised a third group (Table 3).

Table 3. Results of Duncan's Multiple Range Test for PCB Content of Fish From Nine Oklahoma Lakes

Lake	N	Mean PCB (µg/kg)	Grouping*
Ft. Gibson	30	3244	A
Webbers Falls	27	1612	B
Birch	22	430	C
Kaw	30	351	C
Keystone	30	330	C
Oologah	33	240	C
Eufaula	47	154	C
Texoma	91	86	C
Hulah	17	15	C

*Means with the same letter are not significantly different (p = 0.05)

When all samples were grouped by trophic level, detritivores had a mean PCB burden of 781 µg/kg, while carnivores and planktivores averaged 312 and 136 µg/kg respectively (Table 4). Detritivores had significantly greater PCB residues ($p=0.0143$) in their fillets than either carnivores or planktivores. The influence of trophic level on the sample from each individual lake was also tested (with the exception of Hulah Lake which did not have detectable PCB residues in any sample). Detritivores in four of the lakes contained significantly greater ($p=0.05$) concentrations of PCB than the other trophic levels. In the remaining four lakes, either there was no significant difference ($p=0.05$) between trophic levels, or detritivores and planktivores were in a group with significantly greater ($p=0.05$) PCB content than carnivores.

Data from the individual fish species comprising the three trophic levels were analyzed to determine if any species within the levels contained significantly higher PCB residues. Both river carpsucker and carp fillets contained significantly greater ($p=0.0097$) PCB residues than the other species (Table 5). No significant difference ($p=0.05$) was found between the remaining species. The mean concentration of PCBs in fillets ranged from 1099 µg/kg in river carpsucker to 86 µg/kg in white crappie.

Data from selected study lakes was compared by geographical location of the sample to ascertain the influence of sample location within a lake on the resulting PCB burdens. The four lakes; Webbers Falls, Oologah, Eufaula, and Texoma, each had been sampled in two or more widely separated locations, and the samples within the lake were similar in number and composition. No significant difference ($p=0.05$) was found between levels of PCB in fillets and geographical location of the sample for any of the four lakes.

Total PCB content was also compared to fish length (as a measure of age) for each species. Although the size range of the sample had been kept relatively small, no significant correlation ($p=0.05$) was found for any species.

Indicator Organisms

It is probable that mean PCB content of fish from the seven lakes without known sources of PCB input represent "background" levels typical of many Southwestern U.S. lakes. Similar levels of PCB residues in fish from lakes without known PCB sources have been found by the Oklahoma State Department of Health (Oklahoma State Department of Health Report 1981). The results of the present study indicate PCB residue in fish can be related to PCB input into an aquatic system and such information may be sufficiently sensitive to allow differentiation between "normal" concentrations of PCB and elevated levels from either permitted or non-permitted discharges. This represents a recent issue of great importance concerning PCB and the environment; the use of PCB content of organisms as indicators of PCB pollution.

Detecting, evaluating, and regulating PCB sources poses a problem to environmental authorities for several reasons related to the physical characteristics of PCB. Whether the PCB input to an aquatic system is via landwash or effluent discharge, the extremely low solubility of the PCB molecule in water (USEPA Report 1980, Schoor 1975) and its affinity for adsorption to suspended particles insure PCB concentrations in most waters will be low. It may be almost impossible to correlate any PCB found in the water with the magnitude

Table 4. Results of Duncan's Multiple Range Test for PCB Burden in Fish of Three Trophic Levels

Trophic Level	N	Mean PCB (µg/kg)	Grouping[a]
Detritivores	208	781	A
Carnivores	86	312	B
Herbivores	33	136	B

[a]Means with the same letter are not significantly different ($p = 0.05$).

Table 5. Results of Duncan's Multiple Range Test for PCB Burden in Seven Fish Species

Species	N	Mean PCB (ug/kg)	Grouping*	
River carpsucker	41	1099	A	
Carp	52	1055	A	
Channel catfish	47	683	A	B
Smallmouth buffalo	64	414		B
White bass	28	401		B
Gizzard shad	33	136		B
White crappie	42	86		B

*Means with the same letter are not significantly different (p = 0.05)

of the discharge. It is safe to assume most PCB is eventually deposited with sediments when PCB in the water and adsorbed to suspended particles have reached equilibrium. Deposition of PCB in the sediment occurs in response to a variety of factors which include those related to sediment deposition as well as variations in the attraction of PCB molecules to particles. Thus, data obtained from sediments is difficult to interpret and subject to wide variations; the well-known phenomenon of sediment "hot-spots" being an example. At Ft. Gibson Lake, the majority of the contaminated sediments were confined to a small tributary representing no more then 45 ha of sediment. This is only about one-half of 1% of the total surface area of sediments in the lake. Even within the contaminated creek, PCB concentrations ranged from 0.23 µg/g (dry weight) to 7.20 µg/g and were significantly greater at areas of deposition such as the upstream edge of bars and the delta, as compared with the stream bottom and edge (Hunter et al. 1980b).

Increasingly over the past few years, investigators have turned to the biota as a sort of automatic, time-integrating sampling device. Fish are most popular although a variety of organisms from starlings (Sturnus vulgaris) (White 1979) to molluscs (Butler et al. 1978) have been used. An excellent review of the problems associated with this approach has been presented by Phillips (1978). In the Oklahoma data, the lack of significant difference between sample location and PCB content of fillets shows the fish were adequately distributed to provide a representative view of conditions in the lakes. Unfortunately, the standard deviations of many of the samples are quite large, which is a problem common to field observations.

If one seeks to compare this data with data presented by other investigators, two very real problems arise. The first of these is what portion of the body was examined, or whether the entire organism was analysed. Schmitt et al. (1981) acknowledge criticism of the National Pesticide Monitoring Program for using whole fish samples and provides sound reasoning for the choice of whole fish. These include the fact that organisms eat other organisms entirely, and fillet standards designed to protect humans are not particularly relevant in ecosystem studies. Arguments for using fillets, such as ease of preparation and comparison to established standards, are also powerful. It was this latter point, the comparison to established FDA standards, that resulted in fillets being used in the present study. The second problem is whether to express the results on a wet-tissue weight basis or a lipid-weight basis. Although there is some indication various lipid pools within an organism may differentially obtain PCB (Rosewell et al. 1979), reporting on a lipid-weight basis would eliminate much of the variability reported in the literature. These problems are important enough to warrant the establishment of recommended procedures by some authoritative group. The standardization of results to a whole organism (where size permits), fat-weight basis would be extremely useful for comparison of environmental samples and would aid in any future effort towards setting criteria or standards for PCB in organisms.

Assuming steps are taken to collect comparable data, indicator organisms appear to be quite useful in PCB monitoring programs. Careful planning and a knowledge of the ecological relationships of the organisms is required for successful use. The Oklahoma data shows two species, carp and river carpsucker, contain significantly greater ($p=0.0097$) PCB residues than the other fish species. It appears certain organisms or groups of organisms within an

environment will contain significantly greater concentrations of toxic substances than other organisms and this will occur even if biomagnification does not operate. Possible causative factors for this increase are discussed later, but the existence of such species or groups has been demonstrated for PCB (Hunter et al. 1980a,b) and arsenic (Hunter et al. 1981). The identification of these organisms allows monitoring programs to be tailored to trophic levels or individual species which will reduce the variability associated with biological monitoring. This technique has the further advantages of reducing costs and/or allowing increased sampling of the sensitive organisms. Such organisms will vary with geographical region and the toxic substance involved, although there is reason to believe they would be functional equivalents.

A study conducted during 1980 at Webbers Falls Lake provides an ideal example of the usefulness of indicator organisms. Eight sets of water samples were collected at monthly intervals from two depths at each of six locations. No PCB was detected at a detection limit of 0.1 ug/l in any of these 96 samples. Input of PCB to the upper lake had been occurring at up to 2.5 ug/l for four years prior to the study. Twenty-five sediment samples were collected from areas of deposition for 15 miles downstream from the PCB source and analyzed for PCB at a detection limit of 0.3 ug/g (dry weight). None of the samples contained detectable residues of PCB. A second agency also made sample collections during the time of PCB input. Concentrations of PCB in the dissolved and suspended phases of the water were less than the detection limit of 0.1 ug/l in all samples. Concentrations in sediments below the source ranged from less than 1 ug/kg to 16 ug/kg, and averaged 6.2 ug/kg (Stoner 1980). Finally, the group of 29 fish described earlier were collected from the vicinity of the permitted discharge and at a point 13 km downstream. There was no significant difference in PCB content between the two sample locations and PCB content was significantly greater ($p=0.05$) in detritivores than other trophic levels. The total PCB content of the edible portion of the 17 detritivores ranged from 138 to 5940 ug/kg (wet weight). Thus, no information was obtained from the analysis of the water and sediment, but contamination of the biota at levels above surrounding lakes was shown by PCB content of the detritivores.

The identification of groups or species with increased levels of PCB suggests standards or criteria could be set using these organisms as an indicator of excessive PCB input. The present data base is not sufficient to allow recommendation of such a standard, but it could be based on the concentration in the most sensitive indigenous fish species. This is analogous to terminology presently used in many bioassay-based standards. This approach has the advantages of bypassing the insolubility problem of water standards, eliminating the confusion of "hot-spots" in sediment standards, measuring actual levels in the biota, and representing actual processes of mixing and removal in the system. The major disadvantages presently are the previously mentioned lack of a data base and the difficulty in comparing results based on differing measurement and reporting systems. Oklahoma is presently moving in the direction of diversified standards for PCB that recognize some of the problem described above. The 1979 Oklahoma water quality standards designate an aqueous standard and also label concentrations of PCB in fish tissue exceeding 2000 ug/kg as a cause for concern and further investigation (Oklahoma Water Resources Board Publ. 101 1979). Furthermore, the Oklahoma legislature is considering authorization for the establishment of PCB standards based on

sediment concentrations. Such a combination approach may be a method for overcoming the limitations of PCB regulation based on individual components of the environment.

Processes of Accumulation in the Biota

The significant difference in PCB levels between the detritivores and other trophic levels and between the carp and river carpsucker and other species indicates the mechanism producing elevated PCB burdens is related to both ingestion of sediments during feeding activities and adsorption of PCB across the skin. River carpsucker and carp have been found to ingest large amounts of bottom material during their feeding activities; various studies have found sand and detritus in a large percentage of the stomachs of bottom-feeding fish (Wrenn 1968 and Mather 1970). Carp were thought to feed primarily at the mud-water interface by Summerfelt et al. (1970). The smallest, least settleable particles would be found at this interface and PCBs are associated most strongly with such particles (USEPA Report 440/5-80-068 1980). Thus, the opportunity for both PCB partitioning into body lipids and ingestion of contaminated food and sediments would be maximized in this region. While the size range of fish used in this study was relatively small, the lack of correlation between fish length and PCB content of fillets within a species is further indication the mechanism by which PCB is obtained is more complex than mere ingestion. It is probable individual fish come into contact with and ingest PCB laden material in a manner unrelated to age or size. One can hypothesize fish obtaining a base concentration of PCB from water which is related to the lipid content of the individual fish. Another portion of the total body burden of the individual would be ingested in the form of food, sediments, or suspended particles.

The above hypothesis explains the consistent relationship of detritivores, or detritivores and planktivores, containing significantly greater PCB residues than carnivorous species, but only if one assumes biomagnification is not operating. As previously stated, PCB input to detritivores is maximized in their feeding activities. The PCB input to planktivorous species would also be increased through ingestion of PCB-laden particles during filter feeding. However, if excretion is slow (there is conflicting evidence on this point, Califano et al. 1980 and Meier 1982), or at least similar among fish species, than the carnivorous species would contain the greatest levels of PCB if biomagnification occurred. This is especially true for the carnivorous white bass which is rather prey specific to the planktivorous gizzard shad in reservoirs. Differences in lipid content between the trophic levels does not explain this phenomenon. In the subsample from Ft. Gibson Lake, where lipid content was specifically measured, carnivorous species had a slightly higher mean lipid content than the other trophic levels. Furthermore, the lipid content of the samples was not significantly correlated ($p=0.05$) with PCB content of carnivorous species, but was correlated with PCB content in some species of detritivores. This is evidence biomagnification of PCBs does not occur and apparent increases with trophic level are related to interrelationships between levels in the food and environment, feeding habits, and lipid content of the organism.

A recent study (Meier 1982) illustrates the ability of organisms to accumulate PCB. Midges (Chironomus plumoeus) exposed to 1 and 10 ug/g ^{14}C-PCB

contaminated sediments developed significant body burdens of PCB from ingestion of contaminated sediment, and there was a close relationship between substrate concentration and body burden. Fathead minnows fed these midges also accumulated PCB, reaching a maximum within 30 days. The study demonstrated organisms can develop significant body burdens of PCB from the ingestion of contaminated sediments and pass these to higher trophic levels.

PCB IN WILD ANIMAL POPULATIONS

There exists a large data base concerning PCB levels in portions of wild animal populations. A study conducted on the Snake River shortly after the collapse of the Teton Dam found PCB concentrations in rainbow trout averaged 1010 µg/kg. Utah suckers (Catostomus ardens) averaged 1800 µg/kg, and Rocky Mountain whitefish (Prosopium williamsoni) had a mean PCB content of 1400 µg/kg (Perry 1979). A sample of 58 fish from major U.S. watersheds contained PCB residues ranging from not detectable (<300 µg/kg) to 140 mg/kg on a whole fish (wet weight) basis (Veith et al. 1979). About 93% of the samples contained measurable levels of PCB. Commercially harvested mullet from the upper Great Lakes contained PCB residues of 60 µg/kg to 790 µg/kg in edible portions (wet weight) (Zabik et al. 1978).

A series of whole body aggregates of six bottom feeding fish species from Pennsylvania waters contained PCB at 40 to 1630 µg/kg (wet weight). This was substantially greater than similar composites of carnivorous fish which had PCB residues of 50 to 860 µg/kg (Kurtz 1978). Almost 75% of Utah suckers collected from American Falls Reservoir, Idaho, in 1975 had fungus-like growths on the head and sides which the authors attributed to PCB (Kent and Johnson 1979). In that lake, Utah suckers over two-years old were the only fish species with detectable levels of PCB. This was believed to result from exposure to contaminated sediments. An interesting study of the Hudson River used female American shad (Alosa sapidissima) on their spawning run to quantify PCBs. Since the shad do not eat upon entering freshwater, the difference between pre- and post-entry PCB burdens was used as an indication of PCB in the water. Shad from the lower station averaged 2000 µg/kg (whole fish, wet weight) while those from the upper river station had a mean PCB content of 6100 µg/kg (Pastel et al. 1980).

All 206 red-breasted mergansers (Mergus serrator) eggs collected in 1977 and 1978 from Lake Michigan contained PCB at a mean concentration of 22,800 µg/kg (Haseltine et al. 1981). These levels were below concentrations which had an effect on mallard duck eggs (Haseltine and Prouty 1980). Studies of small numbers of crows (Corvus brachyrhynchos), coots (Fulica americana), starlings, and Franklin's gulls (Larus pipixcan) from South Dakota found no PCB at a detection limit of 100 µg/kg (Greichus et al. 1978). In a sample of 350 piscivorous birds of 10 species from a polluted Finnish lake, mean muscle concentrations by species ranged from 220 µg/kg PCB to 13,490 µg/kg. Mean PCB content of the liver tissue from these birds was similar to muscle concentrations (Sarkka et al 1978).

Residues of PCB found in less commonly examined portions of the biota include samples of six earthworms with no detectable PCB (<500 µg/kg), 22 snakes ranging from 1300 to 5800 µg/kg (wet weight), and four nestling birds containing 850 to 1300 µg/kg PCB (Heinz et al. 1980). Harp seals (Phagophilus

groenlandicus) collected from the Gulf of St. Lawrence in 1971 and 1973 contained 70 to 13,300 µg/kg (wet weight) in various tissues (Rosewell et al. 1979). A survey of liver and muscle tissue of mammals collected in southern Ontario found PCB concentrations in 138 fox (Vulpes spp.) and 105 raccoon (Procyon lotor) ranged from 28 to 43 µg/kg. Carnivores such as 25 marten (Martes americana) and 20 mink had residues of 150 to 270 µg/kg, while 15 fisher (Martes pennanti) averaged 600 µg/kg PCB (Frank et al. 1979). The nine eggs of loggerhead turtles (Caretta caretta) collected from Florida all contained PCB at concentrations of 32 to 201 µg/kg. Neither of the two eggs from green turtles (Chelonia mydas) examined contained PCB above the 25 µg/kg detection level (Clark and Krynitsky 1980). Most of the 19 small cetaceans collected from southern California contained PCB at levels associated with reproductive impairment in pinnipeds (O'Shea et al. 1980).

Spatial and Temporal Patterns

The identification of specific geographical areas with increased PCB contamination is difficult due to relative isolation of many sources, movement of the biota, and the impact of improved quantification techniques. Some species of fish from Lake Erie and Lake Saint Clair exceeded Canadian standards, but there was no trend between 1968 and 1976 for the various fish species (Frank et al. 1978). Residues in fish from Lakes Huron and Superior also did not display recognizable temporal trends between 1968 and 1976 (Frank et al. 1978). Concentrations of PCB in Gannett (Morus bassanus) eggs decreased over a six-year period from 7.7 µg/g (wet weight) to 3.5 µg/g (Fimreite et al. 1980). A similar study of white-tailed eagle (Haliaetus albicilla) eggs from Scandinavia showed no decrease in PCB content over a four-year period (Koivusaari et al. 1980). The National Pesticide Monitoring Program has produced a wealth of information relating to spatial and temporal trends of PCB contamination of fish (Veith et al. 1981, Veith et al. 1979, Butler and Schutzmann 1978) as well as other organisms (White 1979, Butler et al. 1978). Although a review of the information obtained in that study is beyond the scope of this paper, two important points can be made. The first is that the majority of the biota of the U.S. is subject to a relatively small base-load of PCB contamination, and populations whose members are completely devoid of body burdens of PCB at modern detection levels are a minority. The second point is most long-term studies indicate a general downward trend in PCB contamination of the biota over the past decade.

CONCLUSIONS

Based on the previous review of literature and original data, the following conclusions and recommendations are made:

1. Bioassay data on PCBs is lacking for large groups of organisms. The use of "standardized" species of bioassay organisms such as fathead minnows and rainbow trout facilitate comparisons between toxic substances but give little indication of the effects of PCB on diverse organisms. Data is particularly needed for large mammals and various trophic levels of birds. Studies of the effects of PCB on progeny should be conducted.

2. There is a constant partitioning of PCB between various components of the environment. Two mechanisms, partitioning into body lipids and ingestion, control PCB residues in the aquatic biota.

3. PCBs are not subject to biomagnification but readily bioconcentrate.

4. The majority of the biota in the U.S. is subject to a relatively small base-load of PCB contamination. Certain organisms, due to their habits, will contain substantially elevated PCB burdens.

5. Small amounts of PCB can inhibit production of phytoplankton, adversely effecting entire aquatic ecosystems. This represents a major threat to the environment and warrants care in establishing discharge requirements.

6. It is probable some U.S. populations of sensitive mammals, such as mink, are being adversely effected by exposure to PCB. These effects would primarily be chronic and related to reproduction but some acute lethal effects are also likely.

7. It is doubtful PCBs are adversely effecting reproduction of fish in most U.S. waters. The more PCB sensitive fish, such as salmonids, frequently have been eliminated by other human actions prior to the use of PCB. PCB levels might hinder reestablishment of such species. Other effects, such as a decrease in the mechanical properties of vertebrae have been shown to occur.

8. Most bird populations in the U.S. would not display acute effects from PCBs in the environment. Many species of piscivorous birds are exposed to dietary levels of PCB sufficient to cause metabolic effects in their offspring.

9. A lack of standardized reporting methodologies has reduced the usefulness of field data on PCBs. The establishment of recommended sample types and reporting methods by an authoritative group is needed. These recommendations should be based on environmental aspects of PCB contamination not strictly related to human health.

10. Determination and regulation of PCB in the environment is made difficult by physical characteristics of the PCB molecule. The relative insolubility of PCB in water and the affinity for adsorption to suspended particles makes correlation of PCB and discharge magnitude difficult. Likewise, deposition in sediments is not strictly related to PCB input.

11. The use of indicator organisms has become increasingly refined over the past few years. It may be possible in the near future to set PCB standards based on concentrations in species which most readily obtain PCB. The process of identifying such species would be greatly facilitated by a central data base such as BIO-STORET.

12. Most long-term studies indicate a downward trend in PCB contamination of the biota over the past decade.

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DISCUSSION SUMMARY

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DR. MELANCON: First, I will make a few brief comments on some of the items discussed by Mr. Hunter. His first slide, which showed some of the published toxicity data for PCBs with phytoplankton and so on, contained data on PCB effects at water levels which are in some instances much greater than the water solubility of PCBs and in most cases greater than levels of PCBs seen in the environment. Rather than mg/l or µg/l concentrations of PCBs typical levels in the environment are ng/l (Pavlov and Dexter 1971), so in terms of environmental lethality of PCBs to phytoplankton other than in isolated instances this is not likely to be a problem.

The second slide showed some data on toxicity tests of PCBs in fish. The concentrations of PCBs which had observable effects were typically higher than environmental levels.

One of the problems experienced by Mr. Hunter in his studies was that the assays for PCBs in water available to him could not quantify PCB concentrations less than 0.1 µg/l. Thus in all of the lakes in Mr. Hunter's studies the PCBs in the water were too low to be quantified, and without the ready availability of more sensitive methods for assaying water for PCBs it will not be possible to tell what role the amount of PCBs in the water has on the results of various studies.

I would now like to describe some work which I am doing and work which has been done in some other laboratories which relate to PCBs in fish in the environment and to some of the items discussed in earlier sessions.

Dr. Friess mentioned that one of the toxic effects of PCBs that was pretty universally found was induction of enzyme activity, and one example he mentioned was induction of monooxygenase activity.

Dr. Safe then related cytochrome P448-type inducers to the 2,3,7,8-tetrachlorodibenzodioxin (TCDD) cytosolic receptor and toxicity. For those of you who are not familiar with induction in fish, fish respond in terms of cytochrome P-450 induction and monooxygenase activity only to P448-type (TCDD-type or 3-methylcholanthrene-(3-MC)type) inducers. So, in other words, of the plethora of PCB congeners only the ones that are the P448-, the TCDD-, the 3MC-type inducers can induce monooxygenase activity in fish.

Thus, if we study induction of monooxygenase activity in fish and get induction it has to have been caused by one of this type of inducer, one of those structures which Dr. Safe has shown as being of the type that binds to the TCDD receptor and presumably somehow causes toxicity in higher organisms.

Another question that was raised this morning was whether anyone can detect any sort of effects resulting from exposure to low levels of PCBs. I would like to present a couple of examples of this.

First, I would like to mention some work that was done by the Department of the Interior jointly by a group from the LaCrosse, WI laboratory and a group from the Columbia, MO laboratory (Bills et al. 1981). They exposed rainbow trout in a flowing system to Aroclor 1254 at levels which were not environmental levels, but which approached environmental levels more closely than most studies. One exposure was at 10 ng/l and the other was at 100 ng/l. After 30 days of exposure the lower level exposure resulted in 0.28 mg/kg whole fish level PCBs, and the higher level gave 2.31. In each case over twenty thousand-fold bioaccumulation in a period of 30 days. They then tested these fish with nine fishery management chemicals and in three or four of those cases they found the toxicity was significantly changed by exposure to PCBs at those levels for one month. So, we have an example of PCB exposure at moderately high levels affecting a biological system.

I would like to mention now briefly some work which I have done that also relates to this. Remember, I mentioned that fish respond with induced monooxygenase activity only to the 3MC-type or P448-type inducers.

In these studies small rainbow trout were utilized for a dose response curve of hepatic microsomal monooxygenase activity in response to a range of doses of Aroclor 1254 administered intraperitoneally (IP) in corn oil. I started dosing at .025 mgs per kg, and increased dosage until the response reached maximum and began decreasing.

The activity peaked at less than 100 mgs per kg, and was well on its way down then.

The enzyme assay utilized was ethoxyresorufin-O-deethylase activity, which Dr. Safe referred to this morning. A significant elevation was found at about 0.3 mgs per kg. This was a single IP injection of Aroclor 1254 at 0.3 mgs per kg and the fish were sacrificed five days later and microsomes prepared by standard methods and the assay done.

We also looked at a different enzyme activity, ethoxycoumarin-O-deethylase activity, and here again at 0.3 mgs per kg there was a significant elevation in monooxygenase enzyme activity.

A similar experiment was done in carp. Sacrificing was done at three days, five days and nine days after IP injection of Aroclor 1254. Here again we observed significant elevation of ethoxyresorufin-O-deethylase activity after a single dose of 0.3 mgs per kg.

Now, why am I doing this? I am interested in induction in the environment, what levels of PCBs and of other pollutants will cause environmental induction in the fish. Now, you remember I brought up the point that the fish respond only to the 3MC-type inducers in Aroclor which we have heard are the types of chemicals that we have to worry about anyway. So, if fish are responding to these very sensitively, this could give us some handle on what is occurring. The point is that in Lake Michigan, which

Milwaukee happens to be right next to, body burdens of PCBs in fish are considerably beyond this 0.3 mgs per kg at which we get significant induction.

I also should mention some other work which has been done at the Medical College of Wisconsin. Dr. Robert Binder, a postdoctoral fellow who is in Dr. Lech's lab has exposed lake trout embryos to PCBs shortly after hatching. He finds that when he administers Aroclor 1254 via the water and gets a body burden of 0.3 mgs per kg he has about a 10-fold induction of monooxygenase activity in the livers of these embryos. Again we see induction of monooxygenase activity at a fairly low level of PCB dosage.

The other side of this research problem, that of demonstrating environmental induction, is something I have started on.

For this particular experiment we maintained some carp in our dechlorinated Milwaukee City water and also pumped Milwaukee inner harbor water into another set of tanks. We maintained the temperatures as similar as possible and they received the same food. After 37 days of exposure the ethoxyresorufin-O-deethylase activity in the carp exposed to harbor water was increased over 10-fold. At 55 days this substantial increase was still present. At 60 days some of the fish were transferred from the harbor water into fresh water, and at the next sampling time at 78 days, the controls stayed about the same, the harbor water exposed were still induced over 10-fold, and the fish which were switched to fresh water dropped to about twice control levels.

The reason I pointed out that part is there have been some papers in the past in the U.S. on assaying fish as a means of monitoring for pollution, particularly by people looking for PAH-type induction. In these studies however they never had controls or a situation where they could take the induced fish back to the lab and show they could get rid of the induction, in other words that it wasn't a strain difference or something like this. There were two experiments done in Yugoslavia (Kurelec et al. 1977, 1981), where they have done something similar to this, and obviously we have shown it with the Lake Michigan inner harbor water. What I would really like to be able to do is to follow this up and relate PCB levels in fish tissues to monooxygenase enzyme activity and actually look for the specific isomers in the fish tissues to relate to this.

May we now have questions from the floor.

DR. BROWN: John Brown, General Electric. I would like to ask a question about the physiological significance to the fish of the monooxygenase induction. I recall seeing accounts in the literature of goldfish having been put in tanks with PCB and absorbing up to 88,000 parts per million in their lipid tissue before they finally turned belly up, and this is many orders of magnitude higher than the levels that you have used in demonstrating enzyme induction.

I also recall at least one study reported with rainbow trout showing a lack of toxicity with dibenzofurans. This would suggest that the correlation between enzyme induction and toxicity that is seen in warmblooded vertebrates may not hold in the fish. Have you observed the correlation with toxicity?

DR. MELANCON: At the levels we are using obviously we don't find lethality or overt toxicity, and that is one of the limitations of just assaying for a single monooxygenase activity. It apparently is a very sensitive indicator and might be of some value in monitoring because of that, but at this point, no, I cannot relate induction of monooxygenase activity to a specific defect which is going to result in the death of the fish or a specific toxic symptom.

One possibility is coexposure to PCBs and other chemicals with subsequent activation of carcinogens and so on but I don't have any toxicity data on this sort of thing.

DR. VODICNIK: Mary Jo Vodicnik, Medical College of Wisconsin. Just to respond to that and to return to reproduction, there are effects showing that PCB injection in rainbow trout results in a decrease in circulating testosterone. This was not related, however, to monooxygenase activity. However, Gustafson's group has shown that PCB administration to rainbow trout results in an in vitro stimulation of androstenedione metabolism (Hansson et al. 1980). So, there may be an effect on reproduction, a physiological effect of induction in fish.

DR. STALLING: Dave Stalling, Fisheries Lab., Columbia, MO. I would like to respond to the comment about dibenzofuran and toxicity insofar as I know, and I think the study you referred to was probably a feeding study by Zitko. The residue work accompanying that study showed as much residue in the fish that were not bolstered as in those that were fed. This study I think just points to the fact that we have no good definitive toxicity data for aquatic species for dibenzofurans; at least if there is, I would love to see it.

To my knowledge we have no data at all for the furans, and there is probably only one study relating to low levels of TCDD exposure. That work was done by Helder and the concentration range there was from 1 to 100 parts per trillion. I think in all cases at 100 parts per trillion they died. Growth and survival were impaired in all treatment ranges, and histopathology was also observed. So, just to set the record straight, I don't think we have any valid data to discuss for furans.

DR. MELANCON: Does anyone wish to make any comments on what the appropriate form of sampling should be to monitor what is going on in fish, whether it should be whole fish, fillet and so on, lipid content or whatever?

DR. STALLING: Dave Stalling, Fisheries Lab. I am not sure I can exactly answer that question. I think the work that has been done on the Great Lakes shows clearly the need to take your samples from a defined length or weight, or minimally from a year class and to keep track of sex. Now, in the National Pesticide Monitoring Program there are 100 stations that are sampled. Due to the press of the matter, in all cases composite samples of five fish each are used so as to reduce variability. It is not possible in that scope, at least with the level of funding associated with it, to do length, weight and age correlation and to get good, valid data, year to year. I think it is imperative that at least the same age class be used and that you keep the growth factors at least well enough in hand.

As far as sampling whole bodies, I think that is based entirely on what you want to do with the data. Regulatory agencies concerned with human health obviously are not too concerned with total body burden, but the predators that we might be interested in assessing effects on don't really discriminate too much between edible portion for human consumption. So, I think that there is a dichotomy that will continue to exist, and it is only when funding or effort permits parallel studies between residues in fillets and whole bodies that we are able to bridge that gap.

I think, minimally in the biological sampling, you should never do specific studies on dynamics unless you have six samples. That is just from our laboratory experience. Three samples are not enough, and I think from a good, statistical design you would probably like to have considerably more. I believe the literature addresses that with regard to what level of residues you wish to discriminate against, and probably to get factors of 30 percent differences in residues you need 30 samples of comparable age, length and weight.

DR. MELANCON: I would like to mention a study done by Dr. Richard Peterson at the University of Wisconsin, Madison in which he compared the rates of elimination of 2,5,2',5'-tetrachlorobiphenyl from rainbow trout and from perch (Guiney and Peterson 1980). We have previously done some work together on rainbow trout, and he found the lipid contents of both species were similar.

He used hatchery fish, not fish caught out in the lake. The $T_{1/2}$s of elimination overall were similar, but the actual tissues within the bodies of the fish which contained certain percentages of lipid were different in the two species. So, if you look just at fillets, the trout looked higher than the perch, but if you looked at the whole body, the differences tended to disappear.

One comment I would make in regard to Mr. Hunter's presentation is that he looked at several trophic levels with crappies and white bass as carnivores, but had no data on the small fish which they could have been eating. Did you by any chance look at any of those?

MR. HUNTER: In a reservoir white bass are fairly prey specific on gizzard shad. So, I think that in the size range we were looking at, I think that those two are directly comparable.

You are correct on the white crappie. I did not look at the minnows. Funding just did not allow me to go into that.

DR. MELANCON: Even in the gizzard shad, the ones you sampled were so big that those could not be the ones they were feeding on. I was wondering what the small ones would have been like? Would you expect similar levels?

MR. HUNTER: I think so, based on the study I mentioned where 80 percent of the burden obtained from the water occurred in the first 12 hours in fathead minnows, I would assume that most of the gizzard shad's burden would come from this source and that it probably would not increase further with age. So, I would suspect that the very young fish would have similar levels to the older fish.

DR. MELANCON: In an experiment with PCBs where 80 percent of final level was reached in 12 hours, I would wonder whether it was a static system and the water level of PCBs was just becoming depleted.

MR. HUNTER: That is a good point.

DR. MELANCON: The other comment I want to make is that in someplace like Lake Michigan where the carnivores are really the top of the food chain and smaller fish like alewives eat smaller organisms which in turn have been down in the sediments, the pollutant which was in the sediment ultimately finds its way into the big carnivores in the lake. Knowing the type of species in a lake and the interrelationships is probably something that should be taken into account when one decides what species to sample and what your ultimate bioaccumulation factor could be and what it could be due to.

DR. BROWN: John Brown, General Electric, again. I would like to comment rather positively on Dr. Hunter's proposal to use the levels in fish as an indicator of environmental contamination and wonder if this should not be pushed even a little bit further than he did. A typical problem encountered in monitoring levels of xenobiotics in the general environment is the extraordinary heterogeneity of the distribution of the agent in different components, particularly in different sediments that may have different distribution coefficients.

It seems to me it is possible for biota that are circulating in the environment to integrate the actual levels over a fair range. You have indicated relatively consistent data for your biota. You have proposed using this as an indicator standard. This is presumably an indicator of environmental contamination, but we are only concerned with environmental contamination in these cases because of its effect on the biota. Perhaps, do you think that this should be a primary standard, rather than a secondary one?

MR. HUNTER: I think that there is going to have to be a lot of work done to bridge the gap between input to the system and levels in the more PCB-sensitive species. It can be done. Also, it can be done for toxic substances other than PCBs. I have seen these relationships in arsenic burdens in fish. They are very different. What you see is very different. It is not in the bottom feeders, but yes, I do, indeed, think that this does have some possibilities.

DR. MELANCON: In a way fish have already been used as monitors. In fact, we know how birds that ate fish and so on were one of the first indicators for some of these pollutants. We had a case in Wisconsin just a couple of years ago where routine DNR fish sampling procedures led to finding some highly PCB-contaminated carp in the Sheboygan River (Kleinert 1978). It was found that on the bank of the river was residue left from a printing firm that had moved out years previously, and every year when the water went up in the spring it leached PCBs out of there, deposited them in the lower part of the river, and any fish that happened to be there at that time acquired large body burdens of PCBs. So, in other words in that case fish served as monitors to find a source of the PCB pollutants.

MR. HUNTER: I think the case at Fort Gibson Lake is another good example of this. The PCB contamination washed in from landflows and contaminated a creek channel, and at most the creek channel held about 45 hectares of sediment surface area. That is about 1/2 of 1 percent of the surface area of that lake, and we never did detect any PCB at the 0.1 micrograms per liter detection limit in the water. So, we could not find it in the water at our detection limit, and going around looking for that 1/2 of 1 percent of the surface area of sediments is kind of like a needle in a haystack and even within the contaminated creek channel the concentrations ranged from 0.23 to 7.2 parts per million on a dry weight for the sediment, and it was significantly correlated with areas of deposition, in other words, the areas at the upstream edge of mudbars and at the delta held more PCB contamination than, say, the stream bottom or the stream edge.

So, again, there is so much variability inherent in sediment sampling that we almost have to use the biota as an integrator.

SUMMARY: It appears that there are advantages in using fish as biological monitors for pollutants such as PCBs. This could be done by using fish to accumulate materials that are waterborne or present in the aquatic sediments with subsequent analysis of fish tissues for selected chemicals or possibly by examining hepatic monooxygenase activity. The choice of species should reflect the monitoring goals such as the types of pollutants to be monitored. Additional studies would be needed to facilitate selection of appropriate fish species for each monitoring goal and additionally might help to provide information on route, duration or magnitude of exposure rather than just indicating that exposure to a chemical of interest (pollutant) has occurred.

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CHAPTER 8

RISK ASSESSMENT

This paper begins by carefully defining the terms toxicant, exposure, and hazard, and relating them mathematically to the term risk assessment. A short overview of PCBs as toxicants, (i.e., compounds which cause acute and chronic toxicity, mutagenicity, fetotoxicity, carcinogenicity, and teratogenicity) follows. The second part of the paper deals with analyses of the acute and chronic human epidemiology data currently available, and the procedures typically used for assessing risk. The risks associated with PCBs and an estimation of the health risks associated with the current environmental levels are discussed.

ASSESSMENT OF THE HUMAN RISKS TO PCBs ASSOCIATED WITH THE EXPECTED ENVIRONMENTAL EXPOSURES

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During the past decades, a combination of "open" systems and sloppy or illegal disposal practices have resulted in the release of large quantities of chemicals into the environment. In the late 1960s, when it became apparent that persistent chlorinated hydrocarbon polychlorinated biphenyls (PCBs) were ubiquitous environmental contaminants which took years to biodegrade, attention was focused on their potential to bioaccumulate to what was believed to be toxic levels in animals and humans. As a result, questions arose regarding the chronic side effects caused by long-term exposure to these chemicals. Reflecting public concern for chemical contamination in general, Congress enacted the Toxic Substances Control Act in 1976, which mandated a phaseout of PCB manufacturing and use. At that time, PCBs were considered a highly toxic and dangerous chemical because of their environmental persistence and their potential for causing permanent adverse health effects, such as cancer.

In recent years, however, a substantial increase in scientific data has resulted in a better understanding of both the actual effects of human exposure to PCBs and the chemically-induced processes leading to cancer. While it was appropriate a few years ago to act on the premise that all potentially carcinogenic chemicals represented similar hazards, recent experience has shown that there are important theoretical and practical differences in the actual hazard each "carcinogen" represents to public health. Similarly, there has been a substantial increase in the amount of data reflecting the toxicity of PCBs in animals and humans. Considering the national concern expressed for environmental contaminants, and in particular for PCBs, it seems both appropriate and necessary to reevaluate the hazard and risks posed by PCBs now that we have more information, and to continue this re-evaluation each time significant changes in our information occurs.

It is the purpose of this paper to provide a framework for identifying the important toxicological considerations used in interpreting the risks inferred by toxicity data, as well as to provide a discussion on the PCB data within this framework so as to arrive at an assessment of the risk to human health.

DEFINITIONS AND BASIC PRINCIPLES OF ASSESSING RISKS

A toxicant is a chemical agent that can produce an adverse effect in a biological system. Such an adverse effect may be an alteration of normal function or destruction of life. This definition is broad since all chemicals are toxic at some dose, i.e., all chemicals are capable of altering some function or producing death in some biological organism. While this statement may seem obvious, it serves to emphasize the basis of risk assessment which is a determination of those circumstances and conditions under which an adverse effect can be produced. As Emil Mrak stated years ago,. "there are no harmless substances; there are only harmless ways of using substances." A chemical is toxic and produces harm only within prescribed conditions of usage.

Risk is defined as the probability that a substance will produce harm under specified conditions (Doull et al. 1980); that is, it is a practical consideration to determine whether or not some harm will be elicited from a specific chemical exposure. Safety is the reciprocal of risk, or the probability that a substance will not produce harm under the specified

conditions. Thus, when determining the risk or safety of a chemical, the critical factor is not necessarily the intrinsic toxicity of the chemical per se, but the likelihood that the level of exposure to the chemical is sufficient to express its intrinsic toxicity.

In general terms, then, the risk is approximated by the equation:

$$R = T \times E$$

where R is risk, T is toxicity, and E is exposure. More accurately, the equation for risk is equal to the toxicity as a function (f) of the exposure, or:

$$R = Tf(E)$$

This better defines the extrapolation, since under certain conditions the risk is not always linear over the entire dose-response curve; and since, depending on how one defines function (f), threshold or nonthreshold dose-response curves may be fitted to this equation. However, regardless of how one chooses to express the risks to chemical exposure, it is clear that: (a) the actual risk is dependent upon both the toxicity (i.e., hazard) and the exposure (i.e., amount of chemical) and (b) to change either alters the risk. Therefore, to make a risk assessment, one must compare the exposure probability with the various dose-response curves for each toxicity to determine whether or not a harmful response is likely to be induced.

In evaluating the risk associated with exposure to PCBs, we will attempt to answer two questions. First, is the level of exposure of a sufficient magnitude such that a hazard exists? Second, if so, what is the hazard or undesirable response expected for that level of exposure. To answer both questions it is necessary to first evaluate the hazards associated with PCBs; i.e., what are the toxic responses and at what levels of exposure will they occur?

Many aspects must be considered and weighed before the hazards to humans associated with PCB exposure can be evaluated. An evaluation of the human hazard and subsequent risk estimation for any chemical must address the data base utilizing a thorough comparative toxicologic assessment. This means that the animal and human data available must be carefully considered in terms of: (a) the breadth and variety of the toxic responses manifested; (b) the degree of species variation or species consistency in the effects monitored; (c) the possible and/or proposed mechanisms of toxicity; (d) the validity of the tests performed and their relevance for extrapolations to man; (e) the dosage used in animal tests versus the expected level of human exposures; and, finally, to the extent that the data are available, (f) the outcomes of serious poisonings and long-term occupational exposures as a guide to the expected human consequences and as a test of the extrapolations made from animal data. Only when a consistent pattern of toxicity in animals coincides with human experiences can accurate and safe guidelines be promulgated.

The following sections of this chapter will discuss the hazard associated with PCB exposure as interpreted from the animal and human data; the choice and use of risk assessment models to define the risk to PCBs from various environmental exposures; the accuracy with which the health risks for a PCB

exposure can be estimated at the present time; and the conclusions reached by this study.

EVALUATING THE ACUTE HAZARD ASSOCIATED WITH PCB EXPOSURE FROM ANIMAL DATA

Several reviews of the mammalian toxicity of PCBs have been published within the last decade (Fishbein 1974, Kimbrough 1974, Peakall 1975, NIOSH 1977, IARC 1978, EPA 1980). Since much of this information has already been given in the previous chapters, a cursory summary rather than an exhaustive review is provided here. This summary presents the types of hazards to be expected and documents the extrapolations one might make to the high exposure human situation based on the animal data. It must first be stated that PCBs have a low order of acute toxicity as measured by the median lethal doses (to 50% of the population) (LD_{50}s) for rodent species. As shown in Table 1, the median lethal doses for various commercial PCB mixtures lie between 1.3 and 19.2 g/kg of body weight. Median lethal doses as high as these classify PCB mixtures, especially the higher chlorinated mixtures, as only slightly toxic chemicals (Doull et al. 1980).

PCBs produce a variety of physiologic changes in animals. For example, in the liver, PCBs increase the amount of endoplasmic reticulum, resulting in enlarged hepatocytes and thereby increasing liver size and weight. Higher doses can cause liver damage and necrosis. PCBs applied directly to the skin of rabbits have produced hyperkeratosis, erethyma, blisters, and desquamation. In primates, oral doses of PCBs have led to facial edema, hair loss, and acne-like pustules, as well as prolonged menstrual cycles and increased bleeding in female animals. Additional symptoms reported in various species include gastric hyperplasia, decreases in red and white blood cells, atrophy of the thymus and spleen, and increases in serum levels of phospholipids, triglycerides, and cholesterol.

However, as with lethality, the doses required to cause these acute effects are generally high. The doses required to cause subchronic effects can also be considered high when the slow rate of metabolism, and therefore a tendency to bioaccumulate, is taken into consideration. While a number of effects have been reported for PCBs, those of the liver (i.e., enlargement and necrosis) are probably the most consistently reported, as are those of the skin (i.e., chloracne or dermatitis). These physiologic responses observed in the liver and skin are fairly predictable effects of many chlorinated compounds.

In assessing the acute and subchronic tests of animals, PCBs do not appear to be remarkably toxic chemicals. The dermal irritation caused by PCBs is not an unusual physiologic response for an organic, solvent-like chemical. The chloracne observed in animals and humans is also a frequently observed effect of other chlorinated chemicals and is reversible in cases where PCBs are the only suspect agent. The observed liver effects (i.e., increased liver weight, proliferation of endoplasmic reticulum, deposition of fat, cell death, and tissue necrosis) are common responses to persistent, chlorinated hydrocarbons, which in general are enzyme inducing agents at lower doses and hepatotoxins at higher doses. Many other chemicals share one or both of these characteristics. While enzyme induction may increase the toxicity of some chemicals and lower the dose producing toxicity, this is not a general phenomenon. In many instances, an increase in metabolism decreases the toxicity

Table 1. Median Lethal Doses for Various Commercial PCB Mixtures

Species	Commercial Brand of PCBs	LD_{50} (mg/kg body weight)
Mouse (oral)	Aroclor 1254	2,000
	Kanechlor	>1,875
Rat (oral)	Aroclor 1221	4,000
	Aroclor 1232	4,500
	Aroclor 1242	8,700
	Aroclor 1248	11,000
	Aroclor 1254	4,000-10,000
	Aroclor 1260	10,000
	Aroclor 1262	11,300
	Aroclor 1268	10,900
	Arcolor 4465	16,000
	Arcolor 5460	19,200
Rabbits (skin)	Aroclor 1221	3,200
	Aroclor 1232	2,000
	Aroclor 1242	1,300
	Aroclor 1248	1,300
	Aroclor 1260	1,300-2,000
	Aroclor 1262	1,300-2,000
	Aroclor 1268	2,500

of other chemicals. Similarly, inducing agents often increase other routes of biotransformation that detoxify toxic chemicals. In fact, enzyme induction is sometimes used therapeutically. Therefore, induction by itself cannot be considered a toxic response, nor can its consequences be accurately predicted in humans.

In monkeys, PCBs cause a conversion of the gastric epithelium that can be described as a dysplastic growth pattern. This does not constitute a neoplastic transformation (Drill et al. 1982). While PCBs have been reported as inhibiting the immune system in mammals, this usually occurs at high doses at which general toxicity also leads to a reduced food intake and thus a reduced nutritional status. In some test species, PCBs provide changes in reproductive performance, but again usually at high or toxic doses. For example, in monkeys and minks, this problem occurs in the female animals at doses clearly producing general toxicity and signs and symptoms of intoxication. Reproductive dysfunction during chemical intoxication is not a particularly unusual finding.

Therefore, it is concluded that the acute toxicity of PCBs in animals is not particularly remarkable, nor does the chemical represent an unusual hazard.

CHRONIC EFFECTS OF PCBs

Mutagenicity

A genotoxic chemical is any compound capable of inducing a permanent, inheritable change in the genetic composition of a cell. Genotoxic effects are divided into three types, depending on the cellular level of the genetic lesion. Mutagenesis refers to mutations or structural changes at the DNA level, called microlesions (Doull et al. 1980). For the sake of discussion, all tests are referred to in this paper as mutagenicity tests. The other two types of genotoxic effects are: clastogenesis, which refers to structural changes at the chromosomal level, and aneuploidization, which refers to a change in chromosomal number. Both clastogenesis and aneuploidization can alter the amount or expression of genetic material; both types of changes are often referred to as macrolesions (Doull et al. 1980).

The "Ames test," which uses mutant strains of the bacteria Salmonella typhimurium that have lost the ability to synthesize their own histidine, is probably the most popular and best known test for mutagenic activity. Since this amino acid is important to protein synthesis, and therefore growth, these cells can grow only if histidine is added to the culture medium. A chemical mutagen can be identified by a mutation in the locus of the histidine defect which reverts the bacteria to the normal state, thus allowing easily identifiable bacterial colonies to grow in a histidine-deficient medium.

Wyndham and co-workers (1976), using the Ames assay, tested the several chlorinated biphenyl mixtures and reported that they were weakly mutagenic. A review of their data indicated that 4-chlorobiphenyl had significant activity. Yet, Aroclor 1221, which has a chlorine content of 1.15 chlorine atoms per molecule and is therefore largely a monochlorobiphenyl mixture, showed a greatly reduced activity. This would seem to indicate that either

the "heavier" PCB molecules were inhibiting mutagenicity or that the activity is greatest for the 4-chloro-isomer of the monochlorobiphenyl compounds. The test results for the polychlorinated biphenyl compounds 2,2',5,5'-tetrachlorobiphenyl and Aroclor 1268 seem to indicate no substantial mutagenic activity. Subsequent attempts to reproduce the Wyndham et al. results have failed (McMahon et al. 1979, Safe 1980, Haddle and Bruce 1977, Schoeny et al. 1979, Levinskas 1981) (see Table 2). The majority of assays reported discount any suggestion that PCBs are mutagenic in the Ames assay including subsequent work by an author of the original observation.

Several studies have demonstrated that PCBs lack the ability to damage or alter chromosomes. Green et al. (1975) looked for significant chromosomal damage in the bone marrow and sperm cells of rats administered the following dosage regimens: a single dose of Aroclor 1242 at 1250, 2500, and 5000 mg/kg; or 500 mg/kg per day for five days. Even though some of the animals demonstrated definite signs of intoxication, there was no evidence of chromosomal abnormalities. These findings are consistent with the results reported by Dikshith et al. (1975) or Garthoff et al. (1981) in rats and the study reported by Hoopingarner et al. (1972) using cultured human lymphocytes. Haddle and Bruce (1977) reported that Aroclor 1254 was negative in the micronucleus test, which measures clastogenic activity (chromosomal breakage) by measuring chromatin bodies in red blood cells. Green et al. (1975), using the dominant lethal test, which measures mutagenic events in germ cells by the embryotoxicity it induces, reported that Aroclor 1242 and Aroclor 1254 gave negative results in rats. Keplinger et al. (1971) and Calandra (1975) also reported a similar lack of mutagenic activity in mice using the dominant lethal test.

Levinskas (1981) has recently reviewed the mutagenicity data for PCBs. Besides the above-mentioned tests, Levinskas discusses many additional, though in several cases less often used, procedures. In his review he refers to a study by Odashima in which Kanechlor 300 and 500 were negative in the standard strains of Salmonella typhimurium used in the Ames assay, as well as several additional bacterial strains. However, Kanechlor 300 was reported positive in one or more of four additional strains, while Kanechlor 500 was listed as negative.

On the basis of sedimentation rates, Stadnicki et al. (1981) have reported that the epoxide of tetrachlorobiphenyl caused single-stranded chromosomal breaks in the DNA of L-929 cells at concentrations ranging from 1 µg/ml to 100 µg/ml. A mixture of two hydroxy metabolites, and to a lesser extent tetrachlorobiphenyl, caused some damage at 20 µg/ml and significant damage at 100 µg/ml. The significance of this test at these high in vitro concentrations is questionable.

Using Drosopholis melanogaster and Bombyx mori as the eukaryote test species, neither the French PCB mixtures, Clophen 30 and Clophen 50, or the Japanese mixtures, Kanechlor 300 and 500, were mutagenic. Odashima (1980, 1981) studied chromosomal aberrations in Yoshida sarcoma cells at PCB concentrations causing 50% growth inhibition: Kanechlor 300 gave a positive response and Kanechlor 500 a negative one. Odashima reported cytogenetic analysis of bone marrow cells in mice exposed to PCBs at a near lethal dose. This test produced the opposite result of the previous study. Odashima listed exposure to Kanechlor 500 as positive and exposure to Kanechlor 300 as negative (EPA 1980, Levinskas 1981).

Table 2. PCBs: Tabulation of Microbial Mutagenicity Tests

Tester strain / Product	S. typhimurium										E. coli	
	C3076	D3052	G46	TA98	TA100	TA10000	TA1535	TA1536	TA1537	TA1538	WP2	WP2uvrA
Aroclor 1268	--	--	--	--	--	--	--	--	--	Neg.	--	--
Aroclor 1254	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	--	Neg.	Pos./ Neg.	Neg.	Neg.
Kanechlor 500	--	--	--	Neg.	Neg.	--	--	--	--	--	Neg.	--
2,2',5-5'-tetrachloro-biphenyl	--	--	--	Neg.	Neg.	--	Neg.	--	Neg.	Neg.	--	--
Kanechlor 300	--	--	--	Neg.	Neg.	--	Neg.	Neg.	Neg.	Neg.	Neg.	--
Aroclor 1221	--	--	--	--	--	--	--	--	--	Pos.	--	--
4-chloro-biphenyl	--	--	--	--	--	--	--	--	--	Pos./ Neg.	--	--

Source: Levinskas 1981.

Norback et al. (1981) reported that Aroclor 1254 transformed C3H10T11/2 cells to Type III foci after six weeks of continuous exposure to 10 ug/mL of Aroclor 1254, while a concentration of 1 ug/mL was negative. Norback and co-workers suggested that the effects of PCBs in culture include promotion. In contrast to this study, Pienta (1981) reported Aroclor 1254 as negative in the Syrian hamster cell transformation test.

Peakall (1981) reported chromosomal aberrations in doves fed a 10-ppm diet of PCBs. The frequency changed from 0.8% in controls to 1.8% in treated birds. The average rate of chromosomal aberrations among treated eggs was higher than the highest control value in only 4 of 17 pretreated embryos. In contrast, PCBs injected into white Leghorn eggs up to concentrations of 20 ppm, while toxic, were not clastogenic.

A thorough review of the mutagenicity tests reveals that PCBs gave primarily negative responses. While some positive results were reported, these were largely in vitro tests and for the Kanechlor brands, which gave inconsistent and mixed results for the 300 and 500 brand mixtures. The relevance of some of the in vitro tests that found positive scores only at concentrations of 10 ug/ml or greater (very high and unlikely in vivo blood concentrations) is questionable. This interpretation is supported by the fact that in many cases a similar experiment gave correspondingly opposite, i.e., negative, results. According to Levinskas, "considering the large number of tests conducted, it is not surprising that an occasional suspicious positive finding resulted, simply on a statistical basis" (1981). A comparison of the number of Ames' tests unable to reproduce the results of Wyndham et al. (1976) supports this contention.

It is the consensus of this review that PCBs do not represent a significant mutagenic risk to humans; a contention borne out or reinforced by the negative animal carcinogenicity studies for PCBs.

Teratogenicity

A teratogen is an agent that produces congenital defects. Although teratogenic responses are generally identified by definite anatomical defects in the offspring, authorities also regard functional or biochemical changes as evidence of teratogenicity.

An important consideration in evaluating teratogenicity data, sometimes referred to as "Karnofsky's rule," is that "any compound administered at the proper dosage, at the proper stage of development, to embryos of the proper species, will be effective in causing disturbances in embryonic development." What Karnofsky's rule calls attention to is the fact that maternally toxic doses capable of killing the fetus or producing teratogenic effects in the offspring are of much less concern than those chemicals causing the same effects at doses far below a maternally toxic level. If the pregnant female is made sick, then the delicate balance between mother and fetus is likewise affected or disrupted and an adverse fetal effect might be expected.

Depending on the stage of the fetus, this disruptive pressure can be manifested in many ways. For example, disease, malnutrition, and stress have all been shown to produce changes in the number and quality of offspring

produced. Even reversible acute effects on the mother's central nervous system, though of short duration, may disrupt feeding enough to cause undesirable responses in the unborn. Therefore, fetotoxicity is of most concern only when it occurs at doses far below those eliciting toxicity in the mother.

The studies concerned with the teratogenicity of PCBs in mice are contradictory. Toeruek (1973) reported that PCBs are not teratogens in mice at dosages up to 500 mg/kg. However, more recent studies have revealed chemically-induced effects in the fetus or in newborns at lower doses. Watanabe and Sugahara (1981) injected mice with Kanechlor 500 at dosages approximating 36 to 182 mg/kg/day on days 6 through 15 of gestation. At the higher doses, the pregnancy rate declined to one third of the control values, the number of resorbed fetuses doubled, and the rate of fetal cleft palates reached 5.8%. The authors stated that since there was no change in maternal or fetal weight gain, the PCBs used had a specific teratogenic effect not due to general maternal toxicity. However, their own results do not support this contention. At the higher dosages, the maternal mortality was higher than the rate of cleft palates among the newborn (12% versus 6%). Also, the authors noted skin lesions, alopecia, and enlarged livers in the dams, indicating significant chemical intoxication.

Marks et al. (1981) reported teratogenic effects for the specific chlorinated biphenyl compound 3,3',4,4',5,5'-hexachlorobiphenyl. At dosages of 2 to 16 mg/kg/day given on days 6 through 15 of gestation there were significant increases in the percentage of resorptions and fetal malformations. Liver damage occurred in the dams and consisted of single-cell centrolobular necrosis and microabscesses. The fetal livers had even a more pronounced injury, including massive necrosis and the formation of a tubule-like pattern of hepatocytes.

Tilson et al. (1979) reported that 32 mg/kg/day of the 3,3',4,4'-tetrachlorobiphenyl, when given on days 10 through 16 of gestation, induced signs of neurotoxicity in the offspring. Some of the newborn were labeled "spinners" because of their increased motor activity and episodes of head bobbing and rotational movements. The spinners had decreased forelimb grip strength and all treated animals had difficulty in crossing wire rods. Subsequent studies (Agrawal 1981) revealed that this exposure level of tetrachlorobiphenyl significantly altered the dopamine binding sites and the dopamine levels in the corpus striatum of newborn mice.

Contrary to Marks et al. (1981), Mattsson et al. (1981) reported that doses of approximately 50 mg/kg/day of 2,2',4,4',5,5'-hexachlorobiphenyl did not increase the resorption frequency, and no fetal effects were noted other than enlarged livers. Likewise, Gellert and Wilson (1979) reported that no reproductive effects were seen in the offspring of mice exposed to Aroclors 1221, 1242, and 1260 at dosages of 30 mg/kg/day on days 14 through 20 of gestation.

Rats have also been extensively studied using dosages up to 100 mg/kg per day. Although these studies indicate that fetotoxicity increases with dose, no teratogenicity has been reported (Keplinger et al. 1971, Calandra 1976, Linder et al. 1974, Villeneuve et al. 1971, Keplinger 1972).

Malformations (terata) have been reported in dogs and pigs in an abstract by Earl et al. (1974). However, the dosages of PCBs used resulted in a severely reduced food consumption in both the dogs and pigs and the authors reported that there may have been an underlying genetic defect that contributed to the teratogenic response in dogs (Drill et al. 1982).

Several studies have been published using monkeys as test species (Allen et al. 1974, Allen and Barsotti 1976, Barsotti et al. 1976, Allen et al. 1980). For six females fed 25 ppm of PCBs for two months, conception was inhibited (Allen et al. 1974). Of the six, only one was able to give birth; all female animals were clearly intoxicated and one died. A second study (Allen and Barsotti 1974) tested 18 female animals fed diets of 2.5 or 5.0 ppm of Aroclor 1248. After seven months' exposure, the eight survivors from each dosage level were bred. All animals on the low-dose diet conceived. There were three resorptions and five births. Of those animals given the higher dose diet, one resorption, three abortions, one difficult delivery and subsequent death, and one normal birth occurred. All newborns were small in size and showed hyperpigmentation, but were otherwise normal (Allen and Barsotti 1976).

In a number of species (e.g., mice, rabbits, rats, and monkeys), PCBs showed no or contradictory teratogenic activity. The study conducted by Earl et al. (1974), still unpublished, provides unconvincing data since it is possible that the malformations observed in the offspring were the result of severe maternal malnutrition (Drill et al. 1982). This conclusion was substantiated by Hansen et al. (1975), who failed to find teratogenic effects in pigs when the mothers were fed a diet of 20 ppm of Aroclor 1242 during gestation and nursing.

PCBs have produced contradictory results in mice. Tests conducted by Marks et al. (1981) and Tilson and co-workers (Tilson et al. 1979, Agraval 1981) indicate that the recorded effects are seen only with symmetrically chlorinated biphenyl compounds and that chlorination in the three and four positions is critical. This may explain why Toeruek (1973) and others (Mattson et al. 1981, Gellert and Wilson 1979) saw no or lesser effects with higher doses of mixtures containing small amounts of the specific isomers or with other hexachlorobiphenyl isomers at higher doses. However, studies by Biocca et al. (1981), like the negative data, greatly lessen the impact of the teratogenic findings in mice. These authors demonstrated that the 28-day LD_{50} for 3,3',4,4',5,5'-hexachlorobiphenyl is about 19 mg/kg/day (ranging between 11 through 33 mg/kg/day). Moreover, extending the observation interval to 50 days, it was found that the dosage levels 10 times lower caused 100% mortality. Disposition studies revealed that the toxicity of the isomers was related to tissue accumulation and that for the 28-day interval the adipose tissue levels at the LD_{50} dosage were approximately 2,000 ppm. These data reveal that the teratogenic effect seen by Marks et al. (1981) occur at doses greater than those that allow for chronic survival. This is consistant with the findings of Watanabe and Sugahara (1981) for which the teratogenic effects of PCBs in mice appear at doses of significant maternal toxicity and lethality. In summary, then, the animal data do not indicate that PCBs represent a significant teratogenic risk at doses not causing significant maternal toxicity. Furthermore, the date suggests that preventing subchronic lethality or chronic organ toxicity would prevent teratogenic effects. Thus, the risk to humans is

expected to be minimal, considering the much lower doses that humans are generally exposed to in the environment and the apparent lack of toxicity expected at these doses.

Studies in animals similarly demonstrate that PCBs represent a minimal reproductive and fetotoxic hazard (for review see Drill et al. 1982). While effects on reproduction or fetotoxicity have been reported, the doses causing such effects are generally high. Rats are fairly resistant, while minks seem to be particularly sensitive. Aulerich and Ringer (1977) reported that minks fed 2 ppm Aroclor 1254 in their diets had decreased reproductive success. The effect at this level was specific for Aroclor 1254 and was not seen for Aroclors 1016, 1221 or 1242. The reproductive impairment was not permanent but consistently occurred at maternally lethal doses. Orberg (1978) has demonstrated an increased metabolism of progesterone, estradiol, and testosterone in animals precreated with PCBs. It may be proposed, then, that doses that greatly increase the metabolism of important hormones could alter the normal hormone homeostesis and result in reproductive problems.

The series of studies by Allen and co-workers (Allen et al. 1974, Allen and Barsotti 1976, Barsotti et al. 1976, Allen et al. 1980) have demonstrated that PCBs may disrupt menstrual cycles and significantly alter reproductive success in monkeys. However, the doses used were clearly maternally toxic, fetotoxic, and toxic to the offspring. Studies similar to those in mice indicate that the body burdens in monkeys at the time of death are quite high (Bailey et al 1980). Since (a) high, maternally toxic or lethal doses were generally required, (b) there are a number of negative studies, (c) the general human environmental exposure is very low, and (d) no reproductive problems in humans have been associated with exposures to PCBs only, PCBs do not appear to represent a significant reproductive risk to humans at the low exposure levels generally occurring via the environment (Drill et al. 1982).

Carcinogenicity

The carcinogenicity of PCBs has recently been reviewed by several authors (IARC 1978, EPA 1980, Levinskas 1981, James et al. 1981, Drill et al. 1982). The following paragraphs provide a summary of the data. Some of the studies reporting the tumorigenicity of PCBs are listed in Table 3. While many investigations have been conducted, only two studies have reported a significant increase in hepatocellular carcinoma. Ito et al. (1974) reported hepatocellular carcinoma in mice after 12 months' exposure to a diet of 500 ppm of Kanechlor 500. Kimbrough and co-workers (1975) also reported hepatocellular carcinoma in female rats fed 100 ppm of Aroclor 1260 for 21 months.

However, contrary to these findings, many similar studies have shown no ability of PCBs to produce cancer. Kimbrough and Linder (1974) found that 300 ppm of Aroclor 1254 failed to produce hepatocellular carcinomas in mice after 11 months. Studies using less chlorinated Kanechlors were similarly unable to produce cancer (Levinskas 1981). Likewise, Calandra (1976), Levinskas (1981), and the National Cancer Institute (NCI) (1978) have reported tests on both Aroclor 1254, Aroclor 1260, and HCB in rats at doses up 100 to 200 ppm; none were found to be carcinogenic. Calandra (1976) has also reported a two-year study of dogs given doses of 1, 10, and 100 ppm that was negative. Studies of rhesus monkeys were also negative (Drill et al. 1982, Allen and Norback 1973).

Table 1. Tumorigenic Effects of PCBs

Authors	Year	Species	Compound, Dosage	Effects
Kimbrough et al.	1972	Rats	Aroclors 1248, 1260, and 1254: 100 ppm/52 weeks	Hypertrophy
Nagasaki et al.	1972	Mice	Kanechlors 300, 400, and 500: 500 ppm	Hepatomas induced by Kanechlor 500 only
Allen & Abrahamson	1973	Rats	Aroclors 1248, 1254, and 1262: 0.1%/6 weeks	Hypertrophy; regressive changes
Ito et al.	1973	Mice	Kanechlor 500: 500 ppm/ 32 weeks	Nodular hyperplasia and well differentiated hepatocellular carcinomas in the liver; PCBs increased hepatic neoplasms in mice and increased the tumors induced by BHC
Allen & Norback	1973	Rhesus monkeys	Aroclor 1248: 300 ppm/ 3 months	Hyperplasia and dysplasia of gastric mucosa with invasion of adjacent tissues
Kimura & Baba	1973	Rats	Kanechlor 400	Neoplastic nodules
Burse et al.	1974	Rats	Aroclors 1242 and 1016: 100 ppm/10 months	Liver cell necrosis
Ito et al.	1974	Rats	Kanechlors 500, 400, and 300: 1000 ppm	Nodular hyperplasia
Kimbrough et al.	1974	Mice	Aroclor 1254: 300 ppm/ 11 months	Hepatomas; adenofibrosis
Makiura et al.	1974	Rats	Kanechlor 500: 0.5% PCBs	Inhibited the induction of liver tumors by several liver carcinogens

Table 3 - continued

Authors	Year	Species	Compound, Dosage	Effects
Nagasaki et al.	1974/75	Mice	Kanechlors 500, 400, and 300: 500 ppm/32 weeks	Hepatocellular carcinoma only for Kanechlor 500
Kimbrough et al.	1975	Rats	Aroclor 1260: 100 ppm/ 21 months	Hepatocellular carcinomas 26/184; hyperplastic nodules 146/184; adenofibromis
Kimura et al.	1976	Rats	PCBs	Anti-promoters in experimental carcinogenesis
Wasserman et al.	1978	Rats	Aroclor 1254: 200 ppm/ 28 months	Hepatocellular adenomas
Levinskas	1981	Rats	Aroclors 1248, 1254, and 1260: 100 ppm/24 months	Modular hyperplasia; hepatoma

Source: James et al. 1981.

PCB mixtures present a distinct problem when attempting to estimate the cancer hazard, and therefore the cancer risk, to humans based on data developed in rodent studies. The crux of the problem is the relevance to place on an increase in rodent liver tumors when attempting to extrapolate the human risk. This dilemma is actually twofold.

First, there has been controversy over the relevance and nomenclature of rodent hepatic lesions. Some of this controversy relates to the criteria used to diagnose cancer. The NCI has recommended a scheme for the classification of hepatocellular tumors and related lesions in rats (Squire and Levitt 1975). This recommendation has not been universally accepted, but is used in some of the reports of PCB carcinogenesis.

The NCI classification scheme is extensive and specific in defining neoplastic lesions. It was concluded by NCI that benign hepatic cell tumors (i.e., tumors without the potential for malignant behavior) could not be diagnosed consistently. Therefore, a term such as "adenoma," which refers to a benign tumor, was not recommended. It was also determined that the term "hepatoma," used to denote a benign liver tumor, was imprecise and thus was not recommended to describe any of the lesions under discussion.

Contrary to tradition, detection of vascular invasion or metastases was not considered by NCI to be essential for the diagnosis of hepatocellular carcinoma. Instead, the use of inclusive cytological criteria describing changes that may or may not have the potential to become the disease process we call cancer, including many responses that will never progress to cancer, was accepted at the expense of the more definitive criteria of malignant or invasive neoplasms. Thus, it seems that the criteria for establishing liver cancer may vary among pathologists.

Only PCBs with a chlorine content of 60% produced cellular morphological changes in rat liver that were classified as hepatocellular carcinoma using NCI guidelines. However, this same PCB mixture was tested in another strain of rat and did not significantly increase the incidence of tumor formation. Several other studies also failed to show any PCB-induced tumors, including a study performed by NCI. Since there is only one report of PCB-induced hepatocellular carcinoma in rats, we must weigh this report against other studies which were negative.

As previously stated, the use of inclusive cytological criteria will define as cancer some hepatocellular changes that will never progress to cancer. Furthermore, the liver changes associated with PCB exposure were reported to regress after the animal has been removed from the chemical exposure (Burse et al. 1974). While some investigators have used the inclusive cytological criteria for classifying hepatocellular lesions, other investigators feel this classification scheme lacks sufficient diagnostic discrimination. Thus, the single report of PCB-induced cancer in rats may use a pathological description that differs from the terminology used for describing the benign tumors seen in the other studies.

The second problem stems from the need to determine the mechanism by which a chemical induces cancer. Chemically-induced carcinogenesis is a special aspect of toxicology, since chemical carcinogens may or may not obey

traditional toxicologic principles. For example, although carcinogens show dose-response relationships, may undergo biotransformation to active or inactive metabolites, and demonstrate specific structure activity relationships as other toxicants do, they may also be unlike other toxicants: they may not demonstrate thresholds and there may exist a long and indefinite latency period between the critical biochemical effect and any cellular or physiologic expression (Doull et al. 1980).

In recent years, the scientific community has concluded that even among chemical carcinogens there are vast differences in the mechanisms by which cancer is induced. Chemical carcinogens can be separated into two groups: one which obeys traditional toxicologic principles and one which does not. Weisburger and Williams, in Casarett and Doulls' Toxicology: The Basic Science of Poisons (1980), separate carcinogens as either genotoxic (initiator) or epigenetic (promoter), the distinction being that genotoxic carcinogens induce cancer by initiating a permanent change in DNA. Epigenetic chemicals comprise those chemicals which alter the manifestation of cancer by other than genetic means and include hormones, immunosuppressants, cocarcinogens, and promoters.

This distinction between types of carcinogens is extremely important (Doull et al. 1980, Squire 1981, Weisburger and Williams 1981), since initiating (genotoxic) carcinogens may cause irreversible damage and may not have thresholds. The concept was generally proposed for all carcinogens early in the last decade and was the basis for developing certain nonthreshold risk assessment models for which some risk is present at any level of exposure. On the other hand, chemical carcinogens that promote cells already predisposed to cancer do have demonstrable threshold levels. Therefore, the risk associated with promoters differs from initiators at low doses in that the risk exists only for the duration that the level of the promoter exceeds the threshold.

Rodents used in cancer tests can have a high spontaneous incidence for liver tumors. Therefore, if a statistically significant increase is seen after chemical exposure, it is important to determine whether or not this phenomenon represents true initiation or merely promotion of the background incidence. Such is the problem for PCBs. To make the distinction between initiators and promoters, the mutagenicity data and other characteristics of the chemical or the carcinogenic response become critically determinant factors.

Initiators are defined as those agents producing damage to DNA which permit cell survival but predispose the cell for uncontrolled growth. Therefore, initiators must possess significant mutagenic activity. There are several other characteristics separating initiating carcinogens and promoting agents; some of these are listed in Table 4. The characteristics of PCBs are also listed in Table 4. It can be seen that the characteristics of PCBs resemble those of a promoting agent and not those of an initiator (James et al. 1981). In his review of the mutagenic and carcinogenic potential of PCBs, Levinskas (1981) likewise cites several authors and experiments supporting the contention that PCBs have promoting activity, including the NCI bioassay (1978).

Table 4. Characteristic Effects of Chemical Carcinogens upon Tissue

Effect	Initiators	Promotors	PCBs
Chemical produces increases of spontaneous tumors at multiple sites.	+	-	-
Chemical increases malignancy.	+	-	-
Chemical produces a tumor capable of transplantation.	+	±	-
Chemical produces a tumor which does not regress once chemical is removed.	+	±	-
Chemical must be mutagenic.	+	-	-
Chemical is affective at nontoxic doses.	+	-	-
Chemical may cause the affect after a single dose.	±	-	-
Chemical should increase the effect of other carcinogens acting on the target organ.	+	±	±
Progression of neoplastic differentiation does not require the continued presence of the chemical.	+	-	-

+ = generally
± = sometimes
- = seldom or never

In summary, when assessing the carcinogenic hazard of PCBs, one must decide what weight to give each portion of the data. While one cannot ignore the fact that positive carcinogenic activity has been reported in rodents, one must take into consideration the weakness of this response since it was not reproduced in several other studies. Furthermore, there is a great deal of evidence to suggest that if PCBs induce changes, it is by way of a promotion mechanism. Thus, on the basis of the animal data and considering the inconsistency of the response, PCBs, as weak promoting agents, at low doses would likely represent a relatively small, if any, cancer hazard in man.

Recognizing the problems associated with accurately interpreting the relevance of and risk inferred by animal carcinogenesis bioassays, Robert Squire, former acting director of the Carcinogenesis Testing Program and head of NCI's Tumor Pathology Section, has proposed a ranking scheme for the animal evidence (Squire 1981). Squire proposes that the basis for determining the strength of a positive test resides in whether the "suspect carcinogen" is positive in a number of species, gives rise to histogenetically different types of neoplasms in one or more species, induces malignant rather than benign tumors, and is consistently positive in an appropriate battery of tests measuring genotoxicity. The dose required to induce tumors as well as the spontaneous incidence of neoplasms in the control animals are also criteria.

By ranking the test data for each criteria according to a point system developed by Squire, one gets an overall score to assess the strength of the evidence. Squire's approach is appealing because it recognizes several features of an animal bioassay that should be considered when attempting to estimate the likelihood that the human response is similar. Since there are several cancer tests in animals for PCBs, the aggregate score that PCBs would receive depends on the weight assigned to the positive and negative data. However, the total score for PCBs, regardless of the scoring, would probably be about 31 or less. Squire classifies a chemical receiving a score of less than 41 as a class V carcinogen, a class for which he suggests that the regulatory options are many and far less restrictive than for chemicals scoring higher in the ranking scheme. Thus, according to this scheme, even the "positive" evidence for PCBs does not rank them as a human cancer hazard that is as significant as the hazard posed by many other chemicals, some of which are still commonly used.

EPIDEMIOLOGY

Acute and Chronic Human Data

In recent studies of occupational exposure, PCBs have not been proven to be a remarkably toxic chemical to persons in the work place, who have probably received the most significant exposure to PCBs. Ouw and co-workers (1976) studied 34 workers from a single capacitor plant using Aroclor 1242. Of this group, 31 were exposed for more than one year and 16 for five years or more. Although industrial hygiene at the plant had been generally poor prior to their study (e.g., air concentrations were 150% to 222% of the allowable workroom air standard of 1.0 mg/m^3), the clinical tests did not identify any significant health problems. One case of chloracne was reported as well as five cases of an eczematous rash. Even though the average PCB blood levels of the group were over 400 ppb compared to none detected in the control study

group, the clinical tests measuring liver function revealed that bilirubin, alkaline phosphatase, serum protein, albumin, SGPT, and immunoglobulins for the group of factory workers were within the normal limits for each test. In addition, bromosulphalein clearance did not correlate well with either PCB blood levels or length of employment.

A more recent study of a far larger group of employees from a capacitor plant reported a similar lack of untoward findings (Fischbein et al. 1979). Based on this study of 326 employees, it was possible to separate the employment records into the following categories for years of exposure: 10% had been exposed for 5 years or less; 20.9% 5 to 10 years; 17.5% 10 to 15 years; 11.4% 15 to 20 years; 29.1% 20 to 25 years; and 11% had been exposed for more than 25 years. A summary of the skin complaints revealed that 10.7% of the employees reported rashes and 24.8% reported a burning sensation of the skin. Physical examination further revealed some redness, swelling, dryness, or thickening of the skin in approximately 50% of the individuals, while 2% experienced abnormal secretions from the eyes.

The clinical chemistry of the study was generally unremarkable, and the researchers commented on the "paucity of abnormal results." Of 321 persons examined, the percentage of samples outside of the normal range were: BUN 5.3%; creatinine 1.7%; SGOT 2.2%; SGPT 7.2%; LDH 2.5%; alkaline phosphatase 1.2%; SGGT 1.9%; bilirubin 0.0%; cholesterol 17.8%; triglycerides 10.5%; and total lipids 3.4%. These data can be considered indicative of the type of random test results to be expected when 321 persons are tested without simultaneously running a control group to compare against the standardized "normal" values used to indicate an abnormal finding. Likewise, the neurologic examinations failed to produce any prevalence of abnormal findings. There was a decrease in the forced vital capacity of the lungs in 14% of these workers compared to only 5.6% in the normal population (Warshaw et al. 1979). While this is an unusual finding, this change was not seen in one group, i.e., the nonsmoking women tested. The significance of this finding is unknown at this time and requires verification from other studies.

Maroni et al. (1981a, 1981b) have reported a study of Italian employees in a capacitor plant exposed to a foreign PCB mixture similar to Aroclor 1242. The mean age and employment of the 80 employees examined was 37 and 12 years, respectively. PCB air concentrations ranged from .048 to .275 mg/m^3, and all tested surfaces were heavily contaminated, indicating that employee exposures were both dermal and inhalation. The results showed that PCB blood concentrations ranged from 88 to 1319 ppb. Fourteen workers were found to have skin disorders consisting of dermatitis, folliculitis, or chloracne. Sixteen employees were reported to have abnormal liver effects, consisting of hepatomegaly and sometimes an increase in serum level of liver enzymes. The liver findings did not correlate well with either the duration of employment or the PCB blood levels, and the pattern for hepatic enzyme changes was a random finding inconsistent with hepatotoxic chemicals. It is evident from these data that the clinical changes were unremarkable. Furthermore, control subjects were not included to determine the normal test variations among unexposed persons of similar ages. Serum bilirubin and alkaline phosphatase levels were within the normal range of variation for all subjects.

Some of the conclusions reached in two recent reviews of the various studies of PCB exposures to date (Gaffey 1981, Brown et al. 1981) are noteworthy. First, while higher exposures to PCBs tend to mean a higher body burden, there was a general lack of correlation between exposure duration and body burden. Second, although not all studies agree, there was also a suggestion that the higher chlorinated PCBs were more likely to accumulate in adipose tissue, suggesting a slower metabolism rate. Third, the general health of the occupationally exposed group was considered good.

In a number of studies reviewed by Gaffey (1981) two of the studies reviewed failed to report clinical findings (other than stating that the workers were in good health) while one measured PCB blood levels of up to 1900 ppb in Finnish workers. Of 15 studies reporting clinical measurements, 11 reported dermatologic effects, 9 reported liver function tests, 6 reported lipid measurements, and 5 reported on blood chemistry. Gaffey (1981) concluded that while there is no clear correlation between PCB blood levels and chloracne, the studies suggest that when PCB blood levels exceed 150 to 200 ppm, chloracne can occur. He also concluded that dermatitis, like chloracne, was a frequently observed effect, and that it may be associated with the higher chlorinated PCB compounds.

Of the nine reported studies of liver function, only five found some mild change in liver function tests. No consistent pattern was identified, nor was an association between these changes and PCB blood levels identified. No adverse health effect was associated with PCB exposure in any of the studies. Of the six studies that considered lipid metabolism, no consistent pattern associates with PCB exposure; one study, by reporting a decrease in all parameters, contradicted all of the studies reporting an increase. Most studies did associate increased triglycerides with the PCB exposure, but the results for cholesterol were inconsistent. No clinical significance has been attached to these findings. Of the five studies of blood chemistry, none reported any relationship between the tests and PCB blood levels. Of the two studies that measured blood pressure, one found no association, while one reported an association between PCB levels and diastolic blood pressure (Gaffey 1981).

In summary, a review of the human data from studies of persons occupationally exposed or whose PCB levels were increased via fish consumption reveals that the combined results of all studies, while demonstrating PCBs caused dermal problems, failed to identify any significant clinical disease associated with electrical grade PCBs. While some physiologic changes were noted, no clinical significance could be attached to any of these, particularly the changes in liver function, which were minimal and generally not compared to an appropriate control group. It has also been noted that PCBs can induce oxidative metabolism in persons occupationally exposed to them (Alvares et al. 1977). Animal studies suggest that inducing agents can increase serum transaminase levels, e.g., SGOT (Whitfield et al. 1972, Martin et al. 1975) and triglycerides (Martin et al. 1975). Thus, the random, minimal increases in serum transaminase levels and increased triglycerides found in persons occupationally exposed to PCBs may represent the predictable phenomena of modest enzyme induction. The National Institute of Occupational Safety and Health (NIOSH) study likewise indicates that chronic liver, nervous, or circulatory diseases are not associated with long-term exposure to levels of PCBs which were much higher than those encountered in the environment.

Therefore, if the environmental exposure to PCBs is insufficient to induce chloracne (the most frequently observed effect), acute, subchronic, or chronic toxicity is probably not to be expected in humans.

In 1968 a mass outbreak of chloracne and other symptoms, later termed "Yusho," was caused by the ingestion of cooking oil contaminated by PCBs and other chlorinated compounds. Higuchi (1976) and Kuratsune (1976) reported that approximately half of the patients had abnormal lipid metabolism. Serum triglycerides were elevated, while cholesterol was not.

Of 43 affected children examined, 23 boys showed transitory decreases in height and weight gain when compared to unaffected children, while 19 girls did not differ from the control group. Also, some of the infants born to women affected by Yusho were small-for-date. Other symptoms in newborns included dark-brown pigmentation, parchment-like skin, eruption of teeth, and larger than usual fontenelles, indicating transplacental passage of PCBs to the fetus during gestation.

Probably the most common symptoms and the ones largely responsible for the identification of Yusho's disease were eye and skin problems. These consisted of acne-like eruptions, follicular accentuation, swelling of the eyelids with discharge from the eyes, and increased pigmentation of the skin. Unfortunately, even though the chloracne was not a permanent condition, it was a discomforting disfigurement that lasted for a period of months to years.

Urabe et al. (1979) have reported on the cause of the deaths of 31 of the 51 persons who died during the first decade after the Yusho incident. Eleven deaths resulted from various neoplasms; a 35.4% cancer rate, which is higher than the 21.1% rate generally reported for that area of Japan. No conclusion can be drawn from this at present, since the Yusho rate was not age-adjusted and further analysis of the Yusho disease must be done at a later date. Of the reported cancers, the types of neoplasms varied. The numbers of cancer for each tissue were: two stomach, one stomach and liver, two liver, three lung, one breast, and two malignant lymphoma.

While the above effects described in Yusho patients give some idea of the possible consequences of overexposure to PCBs, the exact cause of these symptoms is debatable. The type of PCB mixture contaminating the rice oil, Kanechlor 400, has been discovered to contain polychlorinated dibenzofurans (PCDFs) at concentrations as high as 18 ppm (Higuchi 1976, Kuratsune et al. 1972). Measurements of the rice oil used by Yusho patients revealed that it contained PCBs ranging from 1 to 3000 ppm, 5 ppm of PCDFs, and approximately 1000 ppm of polychlorinated quaterphenyls (PCQs) (Kuratsune 1976, Nagayama et al. 1976, Kuratsune 1980). Therefore, it is not known which of the Yusho side effects can be attributed solely to PCBs (Vos et al. 1970) and, if cancer is found in the future to have been significantly increased in this group, the source of cancer induction will remain unknown. (Note: The PCDFs found in the Yusho PCBs are about 1000 times higher than those usually measured in American sources of PCBs). Recently, Kuratsune (1980) reviewed the Yusho episode and suggested that the levels of PCBs and contaminants were actually about half of the amount previously reported. He and others (Kashimoto et al. 1981) have also suggested that since a considerable number of victims did not recover from their symptoms after their levels of PCBs had dropped near normal

levels, the high levels of contaminants still present may be the more likely causal factors.

Reports of high cancer rates among Mobil Oil employees exposed to PCBs (Aroclor 1254) at Mobil's Paulsboro, New Jersey, refinery have been interpreted as indicative of a possible link between PCB exposure and skin (melanoma) or pancreatic cancer (Bahn et al. 1976). The preliminary Mobil study reported eight cancers which developed between 1957 and 1975 among 92 research and development and refinery workers exposed to varying levels of Aroclor 1254 for five or six years in the late 1940s and early 1950s. Of the eight cancers, three were malignant melanomas and two were cancers of the pancreas. According to NIOSH, "This is significantly more skin cancer (melanoma) and pancreatic cancer than would be expected in a population of this size, based on the Third National Cancer Survey." However, it is difficult to draw any conclusions from this study because of the small numbers of individuals exposed and the variety of other chemicals to which they were exposed. In any case, the study was withdrawn for revision and has not yet been re-released (Gaffey 1981).

Of the studies reporting the effects of PCBs on the chronic illnesses or the mortality incidence in humans, only two provide large populations of apparently single chemical exposure. Brown and Jones (1981) conducted a retrospective cohort mortality study of 2,567 employees from two plants where PCBs were used to manufacture electrical capacitors. All workers included in the study were employed for at least three months in areas of PCB exposure. The vital status of 98% of the population was determined and 39,018 person years were accumulated. The types of PCBs used were Aroclor 1016, 1242, and 1254.

The major causes of death for the 163 persons who had died by the time of the report are seperately listed in Table 5. The only statistically significant difference observed was for cancer of the rectum, but only if the female population of plant #2 is considered rather than the total population. However, the plants studied are located in an area where mortality from rectal cancer is greater than the United States average (Gaffey 1981). As mentioned, this unusual increase stemmed from a high number of deaths in the female population of plant 2: similar findings were not reflected in the female population of plant 1 nor in the male population of either plant. While the liver cancer incidence was higher than expected, it was not significant. There was no relationship between duration of employment involving PCB exposure and the risk of mortality due to cancer or cirrhosis of the liver. There was no statistically significant relationship for any cause of death when the total exposed population was considered.

Bertazzi et al. (1981) studied 27 deaths in 1310 workers exposed to PCBs for 20,565 person years. Mortality was studied for the 25-year period between 1954 and 1978 for employment between 1946 and 1970. The study group had been employed for at least six months and the exposure was largely to Aroclor 1254. For all cancers, the observed mortality was 14 versus 5.65 expected. This increase, however, was significant only for the male population. Higher than expected incidences in cancer of the digestive organs and in cancer of the lymphatic or hematopoietic systems were also observed, although these were not significant. There were no reports of liver cancer.

Table 5. Major Causes of Death for Plant Workers Exposed to PCBs: Aroclor 1016, 1242, and 1254

Cause of Death	Observed/Expected	Standard Mortality Rate
	All Causes	
All malignant neoplasms	39/43.79	(89)
Diseases of nervous system	11/12.55	(88)
Diseases of circulatory system	60/62.93	(95)
Accidents	13/18.29	(71)
All other causes	40/44.79	(80)
All causes	163/182.35	(89)
	Malignant Neoplasms	
Cancer of stomach	1/1.66	(60)
Intestine	4/4.03	(99)
Rectum	4/1.19	(336)
Liver	3/1.07	(280)
Pancreas	1/1.90	(53)
Respiratory	7/7.98	(88)
Breast	7/6.84	(102)
Lymphatic	2/4.34	(46)
Other	10/14.78	(68)

Source: Brown and Jones 1981.

In assessing the epidemiologic evidence for carcinogenicity, it must be remembered that in any epidemiologic study it may be impossible to eliminate all of the unrelated but confounding variables in the observed populations. Moreover, it is not uncommon to see measurable increases in one type of cancer over what is mathematically expected, since we cannot choose a subpopulation that reflects exactly the baseline cancer rates of the total population. In fact, it is well known that different areas of the United States have different background rates for many of the specific types of cancer. All this must be considered carefully, and no conclusions should be made concerning any finding of excess cancer which is not statistically significant, but only above expected values, unless this same excess for that specific type of cancer is repeatedly demonstrated in subsequent studies.

A positive correlation between exposure to a chemical and a resultant adverse health effect relies on the following conditions (Doll 1980):

- A positive association must be seen in individuals with known exposures;
- The positive association cannot be explained by bias in recording, detection, or experimental design;
- The positive association must be statistically significant;
- The positive association should show both dose and exposure period dependency; and
- The positive association must be observed repeatedly in subsequent studies and cannot be a single, confounding, variable observation.

To date, the mortality studies concerned with PCB exposures present several problems of interpretation, the most obvious and important of which is the different increases in cancer type reported. Taken as a whole, these studies provide no support for assertions that PCBs are a cancer-causing chemical. The variety of tumor sites found to be of most concern within each study largely differs from the animal data and is inconsistent with the selectivity of currently known promoting agents or initiating carcinogens.

In summary, epidemiologic studies have demonstrated that commercial PCBs are not remarkably toxic chemicals after acute exposure and that, when excess exposure does occur, the usual consequences are dermatologic and not of a serious or permanent nature. Data from investigations involving chronic exposures yield similar conclusions. The majority of studies has not identified a clinical disease associated with PCB exposure, nor has it provided persuasive evidence of health impairment. There is no persuasive evidence of an excess in total mortality or in mortality due to cancer, cardiovascular disease, or nervous system disease associated with occupational exposure to PCBs. PCBs have not been linked to any human cancer. Future investigations of occupationally exposed populations will no doubt clarify this issue as the total mortality within the test group increases with time and yields a larger mortality data base.

PROCEDURES FOR ASSESSING RISK

In attempting to extrapolate the human risk from chemical exposure using animal toxicity as the basis for the risk extrapolation, there are several models to choose from. The model chosen is primarily determined by the type of health hazard of most concern. However, speaking in general terms, there are only two types used. The first type consists of those methods used for extrapolating the human risk directly from the dose for which there was no observable animal toxicity. This method can be applied to most toxicities (except cancer), since thresholds are assumed for these responses. The second type of model is generally used to assess the risk associated with carcinogens. Since many scientists assume no identifiable threshold for this toxicity, there is some risk attached to any exposure. This concept dictates that mathematical models be used to extrapolate to exposures far below those dosages inducing observable responses in the test animal population.

For noncancer toxicities (i.e., threshold toxicities), the models for extrapolating risk are relatively simple and similar to the Suggested No Adverse Response Level (SNARL) proposed by the National Research Council of the National Academy of Science (1980), or the Lowest Observable Effect Limit (LOEL) used by the United States Environmental Protection Agency (EPA) (1980). Basically, this type of calculation is computed by assuming that humans are as sensitive as the test species used. Therefore, the amount ingested by the test animal that gives no toxic response is the safe upper limit of exposure for humans, i.e., the human threshold dose.

The calculation essentially makes the conversion on the basis of the size differential between humans and the test species, usually a body weight comparison rather than surface area. It takes into consideration those factors that affect a truly comparative dose, e.g., absorption factors if the human exposure differs from the test conditions or if the animal dosage regimen differs from the human exposure interval. The calculation is similar to the following:

$$\text{Safe Human Dose} = \frac{\text{TDo.o(mg/(kg)} \times \text{70(kg)} \times \text{A.F.} \times \text{E.R.} \times \text{t1/2} = \text{mg/day}}{\times \text{ S.F.}}$$

TDo.o = threshold, or no observable effect dose in the test species;

A.F. = absorption factor, or ratio of animal absorption divided by the human absorption (absorption animal/absorption human);

E.R. = exposure ratio (dosage regimen animal/exposure regimen human);

t1/2 = ratio of accumulation (half-life in animal/half-life in humans); and

S.F. = safety factor, which depends on the reliability of the data used for extrapolation.

Typically, the safety factor used varies from 10 to 1000, depending on the extent of animal data available and whether or not there is any human data to substantiate the reliability of the number. Of course, the number calculated should use chronic data where chronic exposures are expected. This type of model calculates one value. Exposure at or below this value is considered safe.

The second type of calculation also has several methods or models from which to choose. However, all differ in their basic assumptions or in the mathematical expression used. Hence, at the low exposures in question, their estimation of risk can vary dramatically (OTA 1981). Regulatory agencies currently use the multistage model proposed by Crump (EPA 1980, OTA 1981). Using this model, the risk is linearly proportional to the exposure at low doses. While each exposure is considered to carry some risk, the acceptable risk or safe dose is usually suggested as the exposure range for a 10^{-5} to 10^{-7} risk. This means that daily exposure to that dose would increase cancer by 1 person in 100,000 exposed (i.e., 10^{-5}) or 1 in 10,000,000 persons (10^{-7}) with a lifetime exposure.

Risk to PCBs

As stated earlier in this discussion, the determinant factor for assessing the risks associated with chemical exposure is usually the toxicity (acute or chronic) of most concern that can be expected to occur in humans. With PCBs, as for any chemical, determining the toxicity of most concern is extremely important to setting safe exposure limits. Likewise, determining the toxicity of most concern also determines the public's perception of the harm of overexposure to PCBs, which in turn affects risk assessment. As demonstrated by the types of analysis used, the difference of whether or not a chemical is carcinogenic can translate into magnitudes of differences in the calculated dose considered to be safe.

PCBs are a good example of this dilemma. As seen in the EPA's ambient water quality criteria for PCBs (EPA 1980), the safe dose calculated if PCBs are not considered to be carcinogenic was 210 ug/day; if PCBs are considered to be carcinogenic, the 10^{-5} risk dosage is approximated at 160 ng/day. This is greater than a 1000-fold difference in the "safe" exposure based on the chemical's carcinogenic potential. Thus, determining the carcinogenic potential of PCBs accurately has a large impact on its perceived risk.

Even though many arguments may be made regarding the carcinogenic potential of PCBs, the answer to the question regarding which level may be safe can be found by a simpler, more direct method. While the preceding extrapolations might provide useful guidelines for safe exposures when only animal data are available, they can be and should be tempered by the human experience whenever possible. While human data may not prove that the extrapolations from animal data are correct, they can be utilized in some instances to determine if those extrapolations are incorrect. Such is the case for PCBs.

The model predicting the cancer risk from PCBs based on the data from Kimbrough et al. study (1975) is linear in the dosage range calculated for acceptable risks. Therefore, linear extrapolation to that dose approximating

a 100% cancer incidence in humans using the slope for the curve in that portion of the dose response for low risks provides an overestimation of the actual dose required for a high incidence of cancer (see Figure 1). Performing this extrapolation for PCBs (i.e., 10^{-5} risk is 160 ng/day, so a risk of 1 is 10^{5} x 160 ng/day equals 16 mg/day). At about a 16 mg/day-exposure of PCBs, we should expect a liver cancer incidence of 100% in the exposed population if the low exposure perception of the risk is correct.

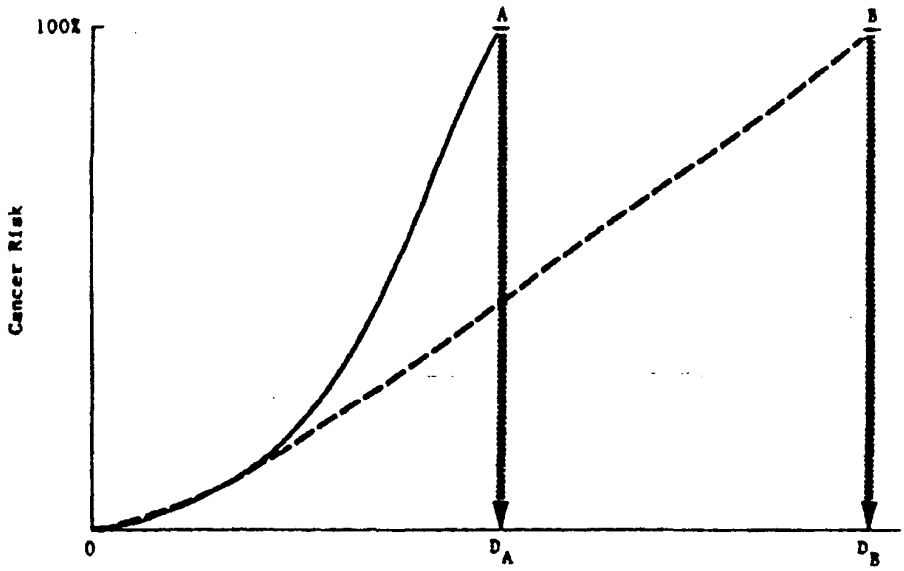
However, the Occupational Safety and Health Administration (OSHA) limit for occupational exposure to PCBs was 0.5 mg/m^3 for Aroclor 1254 and 1.0 mg/m^3 for Aroclor 1242 and other, lower chlorinated biphenyls until PCBs were banned. Assuming that the average volume of air inhaled per workday is approximately 10 m^3, then the allowable workday intake of PCBs could approximate 5 to 10 mg. Granted that perhaps not every worker was exposed daily to these levels, reports such as Ouw et al. (1976) or Brown and Jones (1981) illustrate that it is a reasonable assumption; therefore, we can assume that the daily exposure approximates of our assumption are a practical consideration. Also, the slope of the extrapolated line would be such that the cancer risk above 1 mg/day of PCB exposure would be easy to identify in humans as it is about a 6% of the persons exposed. Since neither the study of Brown and Jones (1981) nor of Bertazzi et al. (1981) found any significant increase in liver cancer, and since neither could demonstrate a consistent cancer risk, one must conclude that the cancer risk extrapolated from the animal data for low dose exposure is wrong and possibly by at least two to three orders of magnitude or greater (depending on the actual relationship between lines A and B in the schematic of Figure 1).

By comparing the risk calculated using animal data to that found in 2,567 capacitor workers (Brown and Jones 1981), we might conclude that one of three possibilities or a combination of all three exists to explain this error in our calculations: (a) the model is wrong, (b) the animal species used does not represent man, or (c) PCBs are not carcinogenic in humans at occupational exposure levels. The first possible explanation may contribute to the problem, but is least likely to be the causal factor for, if reasons 2 and 3 were false, then an increased incidence of cancer in capacitor workers is likely to have been measurable. Therefore, one can conclude that either 2 or 3 or some combination of the three is the more likely reason for the discrepancy. In any case, it can be demonstrated that, at the very least, the results of the animal tests of Kimbrough et al. (1975) do not appear to represent a model or test data relevant for assessing the cancer risk in humans (James et al. 1981). Interestingly, if the modeled cancer risk in the EPA report (1980) is off by only three orders of magnitude, we reach a risk of cancer that is equivalent to the calculated LOEL in this document of 210 ug/day.

It should also be mentioned that this same test of the 10^{-5} risk based upon animal data for DDT and chlordane suggests a large overestimation of the risk for these chemicals as well. Thus, this type of extrapolation seems to be inappropriate for other chlorinated, persistent chemicals which enhance or produce liver tumorigenesis in rodents probably via epigenetic mechanisms.

ESTIMATION OF THE HEALTH RISKS ASSOCIATED WITH ENVIRONMENTAL EXPOSURES

Early in this paper it was stated that a thorough evaluation of the hazards associated with PCB exposures must consider and weigh six different



Legend

A Actual Risk of Data or Model

B Extrapolated risk from low dose region

D_A Actual Dose representing 100% risk of cancer

D_B Extrapolated dose representing 100% risk of cancer

Figure 1. Extrapolation from Low Cancer Risk Exposure to High Cancer Risk Exposure

characteristics of the data.

The first two factors deal with the breadth and variety of toxic responses produced and the degree of species variation associated with the effects monitored. Many of the PCB-induced adverse effects are characteristic of a chlorinated organic compound of the PCB type, or at least these effects are consistently produced by many chlorinated organics. The effects include liver injury, liver enlargement, liver enzyme induction, irritation when applied to the skin, chloracne, and (possibly) a disruption of the estrogenic balance in females due to increased liver metabolism. Although these may indeed be considered adverse effects, all chemicals are capable of altering some physiologic function at a high enough dose. In the more relevant reproductive mutagenic, teratogenic, and carcinogenic tests, tests of chlorinated biphenyls were consistently negative or inconclusive. Some of the studies can be discounted because of inadequate study design, disputable interpretations, or use of unusual test methodology. It is unnecessary to display a disproportionately high concern for those few positive studies when their findings cannot be reproduced in a number of subsequent studies conducted under similar or identical conditions.

Third, regarding mechanisms, sufficient evidence exists to indicate that PCBs have the potential to promote cancer rather than initiate tumors. Similarly, the metabolism and elimination of PCBs in primates is far lower than that of rodents, suggesting that man is far less likely to generate toxic metabolites than rodents, i.e., man is probably less susceptible to liver injury and any impact this may have on tumorigenesis.

The fourth factor regards the relevance of the performed test. For the most part, this was not a factor when considering the results of most tests. There are, however, some problems in attaching the same significance to all of the mutagenicity tests. The human relevance of single-stranded breaks in DNA at high in vitro PCB concentrations or the applicability of the chromosomal tests in birds is questionable when compared to the more often used and better established tests for mutagenic or clastogenic activity in mammalian and bacterial test systems. Thus, we have concluded that the mutagenic, and therefore initiating, activity of PCBs in humans is minimal, i.e., it is not a significant hazard to humans considering the overwhelmingly negative test responses. The relevance of extrapolating one positive carcinogenesis bioassay in rats without balancing it against numerous negative ones in this same species also seems inappropriate considering the likelihood that PCBs are promoters; that the organ response (i.e., liver cancer) has a high background incidence in rodents (Bickerton 1973) and is susceptible to promotion by such effects as induction (Parsino et al. 1973, 1977) and recurrent liver injury (Date et al. 1976, Berenblum 1929, Schumann et al. 1980) (two known effects of PCBs); the species-strain preference of the positive effect; and the irreproducibility of this effect. Considering the promoting pressures that high, chronic doses of PCBs could exert, it is probably more unusual to find a number of responses in rodents negative.

The fifth factor deals with the dosages required to produce animal toxicities as compared to the expected human exposure. In animals, PCBs are acutely toxic only at high doses. Similarly, their chronic effects generally occur at relatively high doses. Thus, taking into consideration the expected

low environmental exposures to humans, PCBs do not appear to pose a significant risk. If skin disorders are not induced, other effects seem unlikely.

The last factor deals with risks determined by human exposures. The epidemiologic studies have demonstrated that PCBs are not remarkably toxic chemicals after acute exposure and that, when excess exposure does occur, the usual consequences are dermatologic and not of a permanent nature. In spite of over 50 years of use, PCB chronic exposures have added little or no additional adverse effects of note to this picture. The majority of studies has not identified a clinical disease associated with PCB exposure other than chloracne, nor has it provided persuasive evidence of an unusual health impairment. There is no consistent evidence of an excess in total mortality or in mortality due to cancer, cardiovescular disease, or nervous system disease that can be associated with occupational exposure to PCBs. PCBs have not positively been linked to any specific human cancer. Therefore, it appears that PCBs are not a remarkable toxicant, but chemicals that require high doses to produce harmful effects.

In evaluating the risk associated with the environmental exposure to PCBs, we should answer two questions. First, is the level of exposure of a sufficient magnitude to constitute a hazard? Jelinek and Corneliussen (1975) estimated that the daily PCB intake for a young male adult dropped from approximately 15 µg/day in 1971 to 8.7 µg/day in 1975. This estimate was based on the assumption that most of the intake was from PCB-contaminated fish. The estimate would not be accurate for people who were not eating such fish, but neither is it accurate for persons who were exposed to waste sites, contaminated water, or other unusual sources. MacLeod (1981) more recently reported that indoor air pollution with PCBs is greater than outdoor air pollution, and often ranges from 0.1 µg/m^3 to 0.2 µg/m^3 of air for many of the locations tested. This approximates to a 1.0 to 2.0 µg/day exposure to PCBs from indoor air. This exposure also approximates the currently recommended NIOSH exposure level for industry. Thus, it appears that persons within the United States are environmentally exposed to microgram amounts of PCBs per day in what are probably the higher exposure instances (excluding unusual conditions). This exposure is 1000 times lower than former occupational exposures, which, according to recent evidence, produced no problems other than chloracne. It would seem that a 1000-fold reduction in exposure would be a sufficient margin of safety for any weak threshold effects that were identified, e.g., skin disorders and induction of liver enzymes. While the NIOSH level is lower than the OSHA standard, it would seem that the old limit reasonably protected the occupationally exposed persons. This bodes well for the rest of us considering our far lower expected exposure from the environment. Furthermore, calculations reveal that PCB exposures of micrograms per day are not likely to significantly alter a person's steady-state tissue concentrations. For example, the average 70-kg man is made up of 15% or greater body fat. In other words, he has at least 10.5 kg of fat in which to store PCBs. Nonoccupationally exposed persons usually contain 2 ppm or less of PCBs in their body fat, thus, at 2 ppm the average 70 kg man would contain 21,000 µg or less of PCBs. If one assumed a worst-case estimate without considering metabolism, excretion, other tissues etc., it would take 28.8 years at a daily exposure of 2 µg/day to double his fat levels of PCBs. At 10 µg/day, it would take 5.8 years; at 50 µg/day, it would take 1.2 years; and at 100 µg/day, it would take 0.6 years. Since the half-life of PCBs is probably no longer than

several months, the actual time to double the body burden is much longer than the above calculations and would never be reached for the lower exposures. Thus, the exposure to PCBs faced by the general population, estimated at about 5 ug/day or less for those exposed, is not likely to have much of an impact upon the low body levels of contamination that have previously been measured in nonoccupationally exposed persons. Since these levels are far lower than the tissue concentrations experienced by capacitor workers, it is unlikely that any untoward consequences can be expected in the general population. The calculations also demonstrate that occasional exposures to much higher levels are not likely to increase a person's adipose levels above the 1 to 2 ppm commonly measured.

From the above discussion, it can be seen that the current daily environmental exposure most likely encountered from the environment is not of a sufficient magnitude to constitute an additional hazard because it is not likely to significantly alter our current body burdens. The animal data has identified certain hazards, but the negative data suggest that these hazards are not consistent or potent responses in every species. While we might speculate that very large doses of PCBs may cause various detrimental effects, the highest exposure group, capacitor workers, appears to have been exposed at levels below those that would cause serious adverse effects in humans. Thus, the current environmental exposures to PCBs, which are much lower than previous occupational exposures, do not appear to be associated with an identifiable hazard, and so the human risk seems to be minimal or insignificant. In order that this paper is not misinterpreted, it is emphasized that this conclusion does not apply to concentrated wastes or chemical waste sites where the exposure may be higher or of a different composite nature. It is also emphasized that higher than microgram-per-day exposures to "pure" PCBs do not necessarily carry an added risk. The Federal Drug Administration (FDA) guidelines of 1 ug/kg/day, or a single exposure of 100 ug of PCBs, is still far below the previous OSHA regulation and occupational exposures or even the EPA's conservative estimate of the LOEL. Until definitive evidence clearly demonstrates a chronic toxicity in capacitor workers or other exposed persons, environmental exposures to commercial grade PCBs (with low levels of PCDFs), which are far less than the former occupational exposure levels, appear to carry a minimal and insignificant risk to humans.

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DISCUSSION SUMMARY

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The considerations of the human risk associated with exposure to polychlorinated biphenyls (PCBs) are varied and require a multidisciplinary evaluation. Risk assessment demands a rigorous evaluation of experimental animal data as well as human exposure data. To accomplish this task, all significant published or readily available studies of the effects of PCBs were reviewed. In addition to this review, current theories of carcinogenesis and toxicity testing and data evaluation were used in evaluating and anthropomorphising the study results. As a result of this review and evaluation, the question was asked, "Does any exposure to PCBs represent a significant health risk to humans?" There was no unanimous agreement among the participants of the symposium as to the answer to this question.

The human risks associated with exposure to PCBs may be considered in two categories, those that are reversible and those that may be irreversible. The discussion began with a question from Ms. Julia Belli (Cincinnati Gas and Electric) about the reversibility of three effects: chloracne, enzyme induction and low birth weights among offspring. In a discussion between Ms. Belli and Drs. Seymour Priess (Drill, Priess, Hays, Loomis & Schaffer, Inc.), James and Harbison, it was brought out that the usual human observable consequence of exposure to excess concentrations of PCBs is a dermatologic condition. The dermatologic condition sometimes produced is chloracne which is reversible and disappears on discontinued exposure. This condition is of an acute nature and chronic exposures have added little or no additional adverse effects of consequence to this picture.

Similarly, the capacity of PCBs to increase liver biotransforming enzymes is not unique to this class of compounds. Although mostly animal studies have been used to determine the effects of PCBs on liver biotransforming enzymes, the effect has also been demonstrated in humans. However, increasing biotransforming capacity of the liver enzymes can have beneficial effects as well as possibly adverse effects. Enzyme induction cannot simplistically be designated a toxic effect. Further, this effect is reversible based upon current knowledge of the biotransforming enzyme systems. Thus, both the PCB-induced chloracne and possible enzyme induction in humans are reversible biological responses.

The implication of any association of causation of low birth weight in human offspring by exposure to PCBs is not supportable at this time. The data presented at this meeting about low birth weight offspring and exposure to PCBs is premature and has not been subjected to a critical review. The low birth weight of some of the offspring exposed to PCBs and other contaminants at Yusho was reversible. Therefore, there are no significant new leads as to the potential for PCBs to produce low birth weight offspring at this time, and

the limited data available show the effect, if it occurs, is transient and does not occur in all offspring.

The controversial but critical issue of assessing the potential risk of developing cancer as a result of PCB exposure, based on studies in experimental animals was addressed. Ms. Jacqueline Warren, of the National Resources Defense Council, expressed concern about two issues: (1) the paper presented by Dr. James generally appeared to review studies that produced positive results more critically than it did similar studies that had negative results and (2) specifically, that a recent National Institute of Occupational Safety and Health (NIOSH) study showed that one group of individuals exposed to PCBs developed a nonstatistically significant three-fold excess of rectal cancer.

Ms. Warren supported her first point by referring to an Environmental Protection Agency review of the study* on which the presentation by Dr. James was based. This assessment, she said, stated that the study's review of the design of the various experiments tended to emphasize and exaggerate the negative effects, while discounting positive effects.

Drs. James and Harbison responded by stating that numerous studies using both laboratory mice and rats were considered. Considering the number of studies which have indicated PCBs cause cancer and comparing this number to the number of studies which failed to demonstrate PCBs cause cancer, the conclusion may be made based on this comparison that exposure to PCBs does not represent a significant risk for increased cancer in humans. Further, after more than 30 years of PCBs use in industry, there is no apparent causal relationship between any type of cancer and exposure to PCBs, and there is no apparent increase in the incidence of cancer mortality among workers occupationally exposed to high levels of PCBs.

The largest study conducted by NIOSH, which involved over 2,500 persons, did not detect any statistically significant excess in the cancer mortality.

Since this study failed to demonstrate an excess cancer rate in a high-exposure population, it provides some reassurance that it is unlikely that future studies will show any increased risk of cancer from PCBs.

The concern for the effects of PCBs on reproduction was expressed by Ms. Warren. She supported that concern by the fact that Allen and co-workers† had shown observed adverse reproductive effects after exposures to concentrations of PCBs equaling 2.5 and 5.0 ppm. She further stated that the concentration of PCBs in human breast milk is in that concentration range or higher.

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*Ecology & Environment, Inc. 1981. Summary of the health effects of PCBs. Buffalo, NY: Ecology & Environment, Inc. Chemical Manufacturers Association contract no. PCB-3.0.

†Barsotti, DA, Marlar RJ, Allen JR. 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Fd. Cosmet. Toxicol. 14:99.

Dr. James stated that the experimental animal studies which were reviewed consistently demonstrated a lack of any teratogenic effect, and fetotoxicity was observed only when maternally toxic doses of PCBs were administered. The experimental animal test results are consistent with the human experience. The Yusho incident, a high PCB exposure, failed to identify any substantive concern for teratogenic effects. Considering the balance of the reproduction studies and the breadth of the mutagenic tests performed, PCBs do not appear to represent a human reproduction hazard.

The overwhelming body of knowledge about PCBs suggests to Drs. James and Harbison that there are some dermatologic effects produced by PCBs, but these effects are reversible, and beyond them there are no clear indicators of any other adverse health effects associated with exposure to PCBs. The human exposure data and the experimental animal data are reassuring in that there have been no significant adverse health effects associated with exposure to PCBs. Therefore, if there are risks associated with exposure to PCBs, they are minimal and unobservable.

Epidemiological studies have shown that commercial PCBs are not remarkably toxic chemicals after acute exposure and that when excess exposure does occur, the usual consequences are dermatologic and not of a serious or permanent nature. Chronic exposures have added little or no additional adverse effects of note to this picture. The preponderance of studies has not identified a clinical disease associated with exposure to PCBs, nor has it provided persuasive evidence of health impairment. There is no evidence of an excess in total mortality or mortality due to cancer, cardiovascular disease or nervous system disease associated with occupational exposure to PCBs. PCBs have not been linked to any human cancer, and studies to date indicate it is unlikely that future studies will establish such a link.

It seems to this author that there was no agreement during the risk assessment discussion as to the consequences of human exposure to PCBs. In fact, Ms. Warren stated that, in her opinion, no meaningful consensus could be reached in the absence of more of the scientists who had performed the studies discussed at the symposium, but who were unable to attend it because of its schedule.

CONCLUSIONS

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INTRODUCTION

This section is a summary of what wee presented and discussed during the two days of the PCBs Symposium. It is not intended to be, and cannot be, a one-man risk assessment of the PCBs problem.

Although I have tried to present information in an accurate and unbiased manner, my professional background and personal bias will undoubtedly flavor these pages. The limitation of such a summary will be further evident by realizing that I wrote it during one day following the meeting, without having had the chance to see the papers beforehand. I did get advice from speakers and discussion leaders in areas outside my own expertise, particularly toxicology, but otherwise had no chance to discuss topics with the authors.

Analytical Methodology

In recent years it became evident that toxicological properties of individual components in the PCBs mixtures show considerable differences. It is therefore necessary to use analytical techniques which allow detection and quantification of, discrete chlorobiphenyls. High resolution capillary gas chromotography (GC), after appropriate cleanup, is the method of choice to fulfill this need.

Capillary GC is no longer a research tool, it has now developed into a viable technique that can be used routinely. There is no apparent strong reason to analyze PCBs in environmental samples by packed column methods. In addition to isomer separation, capillary columns with their much higher resolving power also eliminate many interfering compounds. Quantification of low chlorine PCBs, "early eluters," is difficult if not impossible with other techniques. This problem is also solved by the use of capillary columns.

For proper identification of all compounds in PCBs mixtures and environmental samples, synthetic standards of all 209 chlorobiphenyls are being synthesized, and fully characterized PCBs mixtures are expected to be available as standards in the near future. Several round robin PCBs analyses are being carried out or are planned. One such comparative analysis scheme will use the characterized standards.

For some simple analyses (e.g., transformer fluids, where the chlorobiphenyl pattern has not been changed by environmental or metabolic influences) use of simple packed columns is sufficient, if proper standards are being used.

Gas chromatography-mass spectrometry is still the preferred method when identification aspects are involved. In addition to the time-tested electron

impact ionization mode, negative ion chemical ionization is now available for PCBs analysis.

There is still no standard method for quantification of PCBs. Comparison of several peaks in the sample with a matched PCBs standard is commonly used, but comparison of fully characterized peaks ideally should be used. Preferred detection methods employ the electron capture detector or selected ion monitoring mass spectrometry. Since different chlorobiphenyls vary in their response, particularly in the electron capture detector, work with authentic standards is essential.

The complexities of environmental samples has stimulated the application of pattern recognition techniques (SIMCA) to PCBs analysis. This method establishes similarities and differences between sample residue profiles.

Distribution and Fate

The reduced release of PCBs during the last ten years has resulted in lower PCBs concentrations in the environment. This can be seen by comparing the measurement of PCBs in several organisms made during this time period. The reduction of PCBs content in aquatic organisms is mainly due to burial of PCBs in nonmobile lake sediments and, to a lesser degree, degradation.

Of the total U.S. production of PCBs since 1930, about 15% of the PCBs are believed to have entered the mobile environment, with the northern Atlantic Ocean being the main sink. An estimated 1.7 to 8.6% of the environmental PCBs is in freshwater sediments, making this the second most important PCBs sink. Conversely, the estimated amount of PCBs in freshwater itself is only 0.01-0.04% of the total.

There is a fairly straightforward correlation between degree of chlorination and biodegradability: chlorobiphenyls with a low degree of chlorination are biodegradable, and more highly chlorinated biphenyls are recalcitrant. In one study, monochlorinated biphenyls were shown to degrade with a half-life of days, whereas 2,2',4,4'-tetrachlorobiphenyl showed no measurable degradation. In lake sediments half-lives ranged from a few days for dichlorobiphenyls to 200 days for pentachloro-isomers.

Mathematical fate models now appear to accurately predict the fate, distribution and environmental residues of chlorobiphenyls.

Chlorobiphenyls Incidentally Formed in Process Streams

Although much information and know-how exists for the analysis for commercial PCBs preparations and environmental samples containing such residues, very little is known on procedures for analysing incidentally generated chlorobiphenyls. In the latter case, the relative concentration of chlorobiphenyl isomers is normally related to the individual chemical processes forming them. The chlorobiphenyl content can range from one to several individual components. In virtually all cases, the pattern of isomer and congener distribution is different from that of the PCBs produced by chlorination of biphenyl.

The absence of a characteristic fixed distribution of chlorobiphenyls in some process streams increases analytical problems since, theoretically, all 209 isomers and congeners could be present. The total generation of incidental chlorobiphenyls in the U.S., at concentrations below 50 ppm, is estimated to be 13,800 pounds, of which 95% is disposed of as controlled waste.

Little is known on the actual exposure and the associated hazard from chlorobiphenyls which are formed in process streams and released at low concentrations. Studies on bioavailability are apparently lacking. This information is needed in cases such as the phthalocyanine pigments where chlorobiphenyls seem strongly bound by the matrix.

Bioaccumulation and Pharmacokinetics

PCBs are highly lipophilic compounds that partition into fat and lipid-rich tissues in both fish and mammals. Depending on the degree and position of chlorination, different chlorobiphenyls show different lipophilicities and, thus, different rates of uptake, distribution and excretion.

Uptake of PCBs by fish in their natural environment was shown to be correlated to lipid content in some studies. However, it appears that the concentration of PCBs in organisms is not simply and always related to lipid content, although the studies demonstrating this often suffer from non-standardized reporting methodologies. PCBs concentrations are reported either on a wetweight basis or on a lipid basis. Standardization is urgently needed. Biomagnification (increased concentration in fish of higher trophic levels) is not important for PCBs, but bioconcentration (directly uptake from water) is fast and efficient.

Female rainbow trout and mice were shown to possess alternative mechanisms of PCBs excretion during altered physiological states related to reproduction. Following water exposure of female rainbow trout to 2,2',5,5'-tetrachlorobiphenyl, the half-life of elimination was 1.75 years. Upon initiation of spawning, the half-life of elimination for the same compound decreased to approximately 0.5 years. The half-life of elimination of 2,2',4,4',5,5'-hexachlorobiphenyl from female mice is greater than 100 days. During lactation, however, the half-life from maternal adipose tissue decreased to two days. Within two to five days the mothers had eliminated essentially their entire body burden. The hexachlorobiphenyl, in turn, was stored quantitatively in the offspring. Because of the significantly lower fat content of human milk and comparatively much smaller milk quantities, this mechanism is likely to be much less effective in humans. No significant amount of hexachlorobiphenyl was transferred across the placenta during pregnancy in the mouse experiment.

From analysis of human milk samples and from samples of Yusho patients, it is apparent that the most planar chlorobiphenyls (i.e., those having the least orthochloro substitutions) are the most persistent in the human body. From receptor binding studies it is known that these are also likely to be the most toxic isomers.

Effects on Organisms in the Environment

There has been considerable growth in studies on environmental effects of PCBs in recent years, particularly from a qualitative point of view. Reports of PCBs residue concentrations in limited populations from small areas have been replaced by studies on several components of the ecosystem over larger geographic areas. Acute toxicity studies have given way in many cases to studies of chronic toxicity and to investigations into the mechanisms of toxic action.

Bioassay data on PCBs are still lacking for large groups of organisms. The use of standard species such as fathead minnows and rainbow trout facilitate comparison between toxic substances, but give little indication of the effect of PCBs on other organisms. Data are particularly needed for large mammals and for various trophic levels of birds. Studies on the effects of PCBs on progeny should also be conducted.

The majority of the biota in the U.S. is subject to a relatively small base-load contamination from PCBs. Some organisms, due to their habits, will contain substantially elevated burdens.

Small amounts of PCBs can inhibit production of phytoplankton and could thus have significant ecological effects. It is probable that some sensitive mammals, such as mink, are being adversely affected by exposure to PCBs. These effects would primarily be reproduction-related, but some acute lethal effects are also likely. In many cases, reproduction of fish in U.S. waters is probably not adversely affected by PCBs exposure, since the more sensitive species such as salmonoids have frequently been eliminated by other human actions.

Most bird populations in the U.S. are not expected to display acute effects from PCBs in the environment. Many species of fish-eating birds, however, are exposed to dietary levels of PCBs sufficient to cause metabolic effects in their offspring.

In the past few years, the use of indicator organisms has become refined. It may be possible, in the near future, to establish PCBs standards based on concentrations found in those species which most readily accumulate PCBs.

Human Health Effects

The acute toxicity of PCBs mixtures is moderately low. Despite intense efforts during the last decade, chronic toxicity data for PCBs are still inconclusive. No significant new developments in animal testing were reported during the last few years. In animal studies, great variations are observed in toxicological responses to PCBs and other related compounds. The effects depend on the degree of chlorination in the chlorobiphenyl, the isomer used and the species and sex of test animals. Mink, guinea pig, monkey, quail and chick embryo are among the more sensitive PCBs test organisms, while the rat and mouse are among the less sensitive.

Only a few characteristic responses can consistently be observed in animal testing. These are chloracne and enzyme induction. Furthermore, these

responses can also be observed in workers who are occupationally exposed to PCBs.

Studies using animal models and other test systems have resulted in reported effects which include: (1) adverse effects on the reproductive process (teratogenicity or fetotoxicity in animals), (2) adverse effects on liver tissue in animals with dose related intensity and reversibility, (3) some findings on liver cancer in rats and mice, but otherwise essentially negative results on carcinogenicity, (4) negative findings on mutagenesis, (5) observation of gastric lesions in the monkey after administration of Aroclor 1242, (6) some effects on immuno-competence in animals at dosages that cause changes in nutritional status, and (7) induction of porphyria in the rat and rabbit.

It is clear that more emphasis must be placed on the study of individual isomers. Already, structure-activity relationships in the chlorobiphenyl series, related to the induction of hepatic microsomal enzyme and binding to hepatic cytosol receptors, have yielded significant information. In the polychlorodibenzo-p-dioxin series, these parameters correlate well with toxic effects, and similar, if weaker, responses are observed with the planar chlorobiphenyls (i.e., those having the least orthochloro-substituents on the one hand, and a 3,3',4,4'-chloro-substitution pattern on the other).

It was pointed out that enzyme induction cannot be considered a toxic effect and emphasis should be on meaningful biological end-points. However, apart from being useful in structure-activity studies, enzyme induction could be investigated as a pre-toxic effect marker, since early indicators are essential to community health care.

An internationally approved experiment for testing the carcinogenicity potential of PCBs was suggested. A good study design including both sexes, acceptable species (both rodent and nonrodent) and a carefully worked out protocol could yield significant results. It was also suggested that malignancy and various pre-cancerous lesions should be the indicator of carcinogenicity.

Several cross-sectional epidemiological studies on the health effects of PCBs, and three cohort mortality studies primarily concerned with the carcinogenicity of PCBs, have been carried out since 1978. Evidence from the cross-sectional studies show that occupational exposure was associated with dermatitis and mild liver function abnormalities in the absence of clinical disease. These symptoms were not observed in nonoccupational exposures. The three mortality studies all disagreed on the cancers for which marked excesses were found. The toxic effects observed in Yusho patients is believed to be due to mixed effects from PCBs, polychlorinated dibenzofurans and polychlorinated quaterphenyls. This case should, therefore, not be included in evaluations of exposure effects of PCBs.

Risk Assessment

Sessions on risk assessment are frequently the most difficult and least conclusive in this type of meeting, and the PCBs Symposium was no exception.

In the Chairman's opinion, no meaningful and scientifically sound discussion took place on this topic during the Symposium.

The most worrisome aspect of the PCBs problem is the ubiquitous occurrence of this group of compounds in biological material. This is related to transport behavior, lack of easy biodegradation and the bioaccumulation potential of PCBs (thus, to physical-chemical properties rather than toxic effects). Still, the nagging feeling remains that, perhaps, after 20 to 30 years some as yet unknown or unstudied effect in man or other organisms may become apparent.

On the other hand, it is evident that after 50 years of industrial production and use of PCBs, no significant clinical disease or clear epidemiological correlations have been observed in occupationally exposed persons. Considering the lower and declining exposure to PCBs via the environment, it seems unlikely that significant injury will be observed from environmental exposure to PCBs.

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Monsanto Industrial Chemical Co.

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INDEX