PROPOSAL FOR A HISTORICAL PROSPECTIVE MORTALITY STUDY OF POLYCHLORINATED BIPHENYL EXPOSED WORKERS

BACKGROUND

The polychlorinated biphenyls (PCB's) have attracted much attention since the late 1960's when technological developments in gas chromatography and mass spectrometry made the detection of PCB's at low levels in the environment possible. PCB's have been in use since 1929 in transformers, lubricants, "carbonless" duplicating paper, fireproof sealants, plastics, adhesives, and many other products. Their resistance to biodegradation, photodegradation, and oxidation made them invaluable in scores of uses but also made them more stable as residual pollutants when released into the environment.

PCB's are highly lipid soluble compounds. Lipid solubility enables a proportion of food chain PCB residues to be stored in the fat depots of the body. The first reports of PCB's in the environment came from Sweden, where Jensen (1966)[1] identified residues of PCB's in fish from various Swedish waters. Risebrough et al (1968)[2] found PCB and DDT in marine ecosystems of the Pacific Ocean. Early reports of wildlife effects included egg shell thinning and baby seal effects, but subsequent studies have failed to conclusively show PCB's as the causative agent.

The first report of possible PCB effects on humans occurred in 1968, when over 1,000 Japanese were involved in an epidemic of Yusho, an acnelike skin disease which was traced to rice oil contaminated with PCB's. However, it was later shown that the rice oil also contained a high level of polychlorinated dibenzofurans (PCDF's), possibly due to use of the contaminating PCB as a high temperature heat transfer medium. It was shown that PCDF's are relatively more concentrated in the liver,

and the investigators' findings clearly indicated the necessity to pay greater attention to PCDF's for clarification of the nature of Yusho.[3] While acute effects from relatively high PCB exposure levels are known and can be avoided by appropriate control measures, attention has more recently been focused on the chronic effects resulting from long-term low levels of exposure to PCB's.

Animal tests have proved to be equivocable. The Environmental Protection Agency considers PCB's to be suspect carcinogens based on its own animal tests. Monsanto's extensive two-year chronic oral toxicity tests of Arochlor 1242, 1254, and 1260 in both rats and beagles at dose levels of 1, 10, and 100 ppm concluded that PCB's were non-carcinogenic. Hyperplasia, hypertrophy, and several benign tumors were observed in the livers of the rats, but there was no evidence of malignancy in any of the test animals.[4]

Other investigators have shown liver tissue effects ranging from hyperplasia to well-differentiated hepatocellular carcinomas in rats or mice fed with various PCB-containing diets. For example, Kimbrough et al (1972)[5] fed rats with Arochlor 1254 and 1260, and found hypertrophy, lipid accumulation, and adenofibrosis in liver tissue, but found no malignancy. The same investigator fed Arochlor 1254 to mice for 11 months and reported the induction of malignant hepatomas in the test animals.[6]

The recent National Cancer Institute report [7] of its carcinogenicity tests on Arochlor 1254 concluded that the substance was <u>not</u> carcinogenic in Fischer 344 rats under the conditions of the bioassay. However, they allude to the non-significant occurrence of gastrointestinal tract carcinomas and hepatocellular proliferative lesions in their test animals as possibly being related to the Arochlor diet.

There have been very few epidemiological studies of human populations

exposed to low levels of PCB's over a long period. The Mobil Oil Company released a study in 1976 conducted by Dr. Anita Bahn[8] in which a significant number of melanomas and pancreatic cancers were found in exposed chemical workers. While these results received publication, the study was severely criticized[9] due to deficiencies in the identification of the study cohort, failure to identify other potentially carcinogenic chemicals to which the workers and research personnel were exposed, and other problems which left the results of the Mobil study highly in doubt.

A preliminary study of PCB-exposed workers was begun at Monsanto shortly after the Mobil study in response to the need for a valid epidemiological study of the effect of PCB's on humans. The study focused on workers at the Krummrich Plant, where PCB's were manufactured from 1936 until production ceased in 1977. No melanomas or pancreatic cancers were found, but an excess incidence of lung cancer deaths was seen. However, due to the preliminary nature of the study, several necessary steps to complete the epidemiology study remained unfinished, such as following up on the mortality experience of over 30% of the study cohort who were lost-to-follow-up. In addition, no minimum limits on exposure time were used in the preliminary study. Thus, no attempt was made to distinguish between the workers exposed for years and the worker exposed for a few days.

The investigators involved in the preliminary Monsanto PCB study realized these deficiencies and advised that the study population should be further defined and evaluated so that a valid, comprehensive report could result. Therefore, due to the preliminary nature of the epidemiological data available and the inconclusive toxicological data, it is necessary to pursue further epidemiologic study in order to evaluate the chronic effects of PCB-exposure to man with more complete vital information on a verified, exposed worker cohort.

PURPOSE OF THE STUDY

This epidemiological study will investigate the mortality experience of workers exposed to polychlorinated biphenyls in their manufacture. Through studying this cohort we intend to (1) identify groups of workers that have PCB exposure of a sufficient latency to demonstrate any chronic health effects, and (2) analyze the mortality experience of these workers with attention given to malignant neoplasms especially of the lung, liver, skin, and pancreas.

STUDY POPULATION

The Monsanto plants involved in the U.S. manufacture of PCB's are the W.G. Krummrich plant in Sauget, Illinois, and the Anniston, Alabama plant. Since the preliminary study, upon which the proposed study is based, involved the Krummrich plant alone, the proposed study will deal solely with the Krummrich workman population.

The study population from the Krummrich plant consists of all male waged employees who have worked at least six months in a job involving exposure to PCB after December 31, 1944, and prior to December 31, 1965. This population includes active, retired, and terminated employees. Through interviewing plant personnel, it was determined that exposure levels of workers involved in the manufacture of PCB's did not vary considerably; therefore, all waged workers involved in the manufacture of PCB's will be considered to have a common exposure level.

Work histories for all past and present Krummrich employees have been obtained and screened for PCB-department work by the Department of Medicine and Environmental Health (DMEH). A plant-developed computer listing of all PCB department workers has also been supplied to the DMEH, and the computer listing has been cross-checked with the hand work history search in order to insure completeness. It should be noted that some workers, e.g., maintenance, may have had significant exposures to PCB's, but are not included in the study due to exposure verification difficulties.

Salaryintern w/ 246 females.

For each worker the following information has been obtained: name, social security number, employee number, race, sex, date of birth, date of hire, date of termination (if applicable), and as detailed a work history as is available. For those known to have died, the date and place of death, and a copy of the death certificate are being obtained.

MORTALITY FOLLOW-UP

1. All active employees, and retirees who are currently receiving pension checks will be assumed to be alive as of December 31, 1977.

2. All persons known to have died on or after January 1, 1978, will be assumed to be alive for the purposes of this study.

3. Personnel folders and pension files will be searched for death certificates or for pertinent information about the decedent if no death certificate is present.

4. The names of persons for which there is no information on vital status will be reviewed with plant personnel for any information which they can provide.

5. Other follow-up procedures, such as Veteran's Administration files, Polk Directories searches, telephone directories searches, and state driver's license files will be used, if necessary, to determine the vital status of lost-to-follow-ups.
6. A state death certificate will be obtained for every person known to have died.
7. An experienced, trained nosologist will review and code all death certificates for the underlying cause of death to the International Classification of Diseases, Seventh Revision.

ANALYSIS

The data will be analyzed using the modified life-table method. In this method of analysis,the age, race, cause, time-specific mortality rates in a standard population are applied to person-years classified by age, race, and time-specific subgroups of the worker population at risk (i.e. exposed) over the years of observation intervals. A standard mortality ratio will be calculated as the ratio of the sum of the observed deaths to the sum of the expected deaths x100.

The person-years of observation will be calculated by year of observation and age at observation as shown in Appendix A. Person-years of observation will be accumulated from date of first exposure to PCB's to the earliest of the following events: 1) date of death, 2) December 31, 1977, the cut-off ascertainment date, or 3) date at which the study member was lost-to-follow-up.

The expected number of deaths will be calculated using United States annual mortality rates, or county rates, if available, for every year from 1945-1977. If annual county mortality data is unavailable, the 1950-69 expected numbers of cancer deaths based on U.S. rates will be adjusted by cause for the local county death rates according to the ratio of the local county rate to the U.S. rate using U.S. Cancer Mortality by County: 1950-69. A standard mortality ratio will be calculated for certain selected causes of death (Appendix B).

David C. Musch

David C. Musch

APPENDIX A

PERSON-YEARS OF OBSERVATION BY YEAR OF OBSERVATION AND AGE

AGE	1945-49	1950-54	1955-59	1960-64	1965-69	1970-74
15-24						
25-34						
35-44						
45-54						
55-64						
65-74						
75-84						
85 +						

MONS 056206

APPENDIX B

Krumarich PCB Study

Cause of Death with I.C.D. Number (Seventh Revision)	Observed	Expected	P.M.R.
Malignant neoplasms (140-205)			
Buccal Cavity and Pharynx (140-148)			
Digestive Organs and Peritoneum (150-156A, 157-159)			
Biliary Passages and Liver (155-156A)			
Other Parts of Digestive System (Residual)			
Respiratory System (160-164)			
Bronchus, Lung, and Trachea (162-163)			
Other Parts of Respiratory System (Residual)			
Genito-Urinary System (177-181)			
Leukemia and Aleukemia (204)			
Lymphosarcoma and Other Neoplasms of Lymphatic and Hematopoietic Tissues (200-203, 205)			
All Other Neoplasms (Residual)			
Diseases of the Cardiovascular System (330-334, 400-468)			
Diseases of the Respiratory System (470-527)			
Diseases of the Digestive System (530-587)			
All Other Diseases (Residual)			
All External Causes (E800-E998)			

MONS 059207

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FINAL REPORT OF THE
SUBCOMMITTEE ON THE HEALTH EFFECTS
OF
POLYCHLORINATED BIPHENYLS
AND
POLYBROMINATED BIPHENYLS

JULY 1976

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

WASHINGTON, D.C.

Attachment 1-7

MONS 056209

ERRATUM - Table numbers proceed from 9 to 11. There is no Table 10.

MONS 056210

CONTENTS

MONS 056211

MONS 056212

MONS 056213

MONS 056214

SUBCOMMITTEE ON THE HEALTH EFFECTS
OF
POLYCHLORINATED BIPHENYLS
AND
POLYBROMINATED BIPHENYLS

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Food and Drug Administration

Dr. Frank Cordle (M)
Food and Drug Administration

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Food and Drug Administration

Dr. David Firestone (M)
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MONS 056215

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MONS 056216

Dr. Richard H. Teske (M)
Food and Drug Administration

Dr. William Trotter (M)
Food and Drug Administration

MONS 056217

SUBCOMMITTEE ON THE HEALTH EFFECTS
OF
POLYCHLORINATED BIPHENYLS
AND
POLYBROMINATED BIPHENYLS

Chairperson: A. C. Kolbye, Jr., M.D.

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2. Metabolism and Biochemical Toxicity

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3. Animal Toxicity and Carcinogenesis

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R. Teske

4. Human Exposure

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MONS 056218

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J. Moore
R. Shapiro

MONS 056219

Subcommittee on the Health Effects

of PCBs and PBBs

Purpose:

1. "Assemble, review and interpret data that assess the health significance of polychlorinated biphenyls."

2. "Formulate recommendations as to future research needs."

Specific Objectives:

1. Chemistry:

   Assess the impurities that may be found in PCBs and PBBs, the "hardness" of the data giving their potential for accumulating in the food chain.

2. Metabolism and Biochemical Toxicity:

   Assess the Metabolism of PCBs and PBBs and the possible contribution of specific isomeric homologs to the observed toxicity; evaluate possible effects at the cellular level; and define existing dose-response relationships for the phenomena.

3. Animal Toxicity and Carcinogenicity

   Assess the short and long term effects, including carcinogenis, of exposu.. to PCBs and PBBs in various animal models, their relationship to known human effects, and the establishment of dose-response curves.

x

MONS 056220

4. Human Exposure

Quantity known human exposures, accidental pulsed, and long range, their relationship to possible overt symptomatology, and the establishment of possible dose-response curves.

MONS 056221

INTRODUCTION

PCBs were reportedly first synthesized in 1881 by Schmidt and Schultz, but were not available commercially until 1930[1] (Hubbard, 1964). From 1930 until 1970, when their distribution in the U.S. was voluntarily restricted by Monsanto to closed systems, the use of PCBs in a wide variety of industrial applications had steadily increased.

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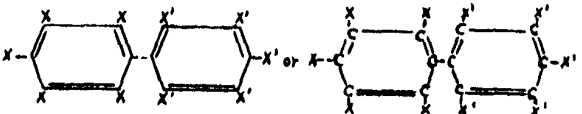
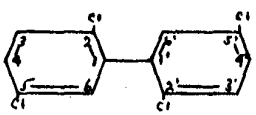
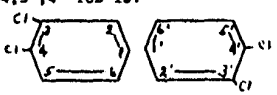
1. Commercial PCBs are complex mixtures. Arochlor 1254, for example, contains 69 different molecules (Zitko, 1971), which differ in the number and position of chlorine atoms.



This is a chemical representation of a PCB molecule. C = carbon atom, X = Cl (chlorine atom) or H (hydrogen atom). The basic parent molecule contains two benzene rings connected by a carbon-carbon bond. When all the X' and X are hydrogen, the molecule is called biphenyl. When several of the hydrogen atoms are replaced by chlorine atoms, the molecule is called a PCB. X and X' are used to indicate that the chlorine atoms are on different benzene rings. For example, the chemical structure for 2,5,2',5'-tetrachlorobiphenyl is:



Positional isomers have the same number of chlorine atoms, but they are located at different positions on the ring. 2,5,2',5'-tetrachlorobiphenyl and 3,4,5',4'-tetrachlorobiphenyl (TCB) are isomers. They are different molecules with different chemical, physical, and biological properties. The chemical structure for 3,4,3',4'-TCB is:



MONS 056222

The problem of polychlorinated biphenyls (PCBs) became of national concern in 1971, when several accidental contaminations of foods were reported. In addition, the extent of the environmental contamination, and its persistence, were not precisely known. Subsequently, various regulatory actions were taken by the concerned agencies involved and, with the cooperation of the only U.S. producer, the situation was felt to be under control.

Then, in 1975, the reported high levels of contamination, with PCBs, of Hudson River fish, refocused national attention on this environmental contaminant. It was soon apparent that the actions and control measures of the early 1970's had not succeeded in totally reducing, or even substantially alleviating the problems associatad with this environmental pollutant.

On November 17-19, 1975, a National Conference on Polychlorinated Biphenyls, sponsored by the Environmental Protection Agency in cooperation with the Department of Agriculture, Council on Environmental Quality, Department of Health, Education and Welfare and Department of the Interior, was held in Chicago, Illinois. This confsrence brought together the latest data, and scientific expertise, to consider all aspects of the various problems associated with PCBs, and the means for their possible resolution.

In his introductory remarks to the Session on Health Effects and Human Exposure, Dr. David P. Rall, Session Chairman, announced the formation of a Subcommittee on PCBs of the DHEW Committee to

MONS 056223

Coordinate Toxicology and Related Programs. The Subcommittee was given the charge to:

1. Assemble, review and interpret data that assesses the health significance of polychlorinated biphenyls.

2. Formulate recommendations as to future needs.

The accidental contamination of animal feeds with polybrominated biphenyls (PBBs), and consequent human exposures, that occurred in the State of Michigan, has been approached not only on its own basis, but in comparative relationship to the PCBs, as well. Therefore, the scope of this Subcommittee was expanded to the Subcommittee on the Health Effects of PCBs and PBBs. Members of this Subcommittee came from within the Department of Health, Education and Welfare, as well as including consultants from other Federal Agencies concerned with this difficult problem.

The report which follows represents the culmination of this Subcommittee's efforts.

GENERAL
SUMMARY AND CONCLUSIONS

CHEMISTRY

The great complexity of PCB commercial mixtures has provided the difficult task of separating the components of these mixtures and determining the complete identity of the compounds. Efforts to define these mixtures have been quite successful over the past several years (Hutzinger et al., 1974). Identification of the component chemicals in PCB residues presents another difficult problem. Little progress has been reported in this area. Since the fate of components of commercial PCB mixtures entering the environment cannot be followed directly, inferences must be drawn about chemical alteration of chlorinated biphenyl compounds in the environment mainly based on laboratory studies.

The known resistance of aryl chlorides to chemical oxidation and hydrolysis presumably was a major factor in the promotion of PCBs for industrial uses. Exposure to non-metabolic environmental agents is unlikely to result in significant oxidation or hydrolysis of chlorinated biphenyls. However, since appreciable photoalteration of chlorianted biphenyls has been observed using either sunlight or sunlight-simulating lamps in the laboratory (Hutzinger et al., 1974) as energy sources, it is highly probable that photochemical changes occur in the environment. These photochemical studies have indicated that reductive dechlorination to biphenyls

MONS 056225

of lower chlorine content is a predominant alteration route although formation of more polar compounds, including a chlorinated dibenzofuran, has also been reported (Hutzinger et al., 1974; Crosby and Moilanen, 1973). The diverse conditions of the environment make it difficult to predict accurately the end products and rates of photoalteration of chlorinated biphenyls. From a comparison of carbon-halogen bond-energies, brominated biphenyls might be expected to be more readily altered by light than chlorobiphenyls. But because of the lower volatility and different end uses of bromobiphenyls, they may not be as readily exposed to light as the chlorinated analogs.

A typical PCB residue from fish resembles the Aroclor 1254 mixture more closely than it does other Aroclors (Zitko et al., 1972; Veith, 1975). Considering the major components of Aroclor 1254 (Sissons and Welti, 1971) it appears that penta-, hexa-, and heptachlorinated biphenyls tend to concentrate at this trophic level in the biosphere. It is of considerable interest, therefore, to carry out comparative accumulation, metabolism and toxicity studies emphasizing these higher chlorinated biphenyls using compounds of known chlorine substitution pattern. Individual chlorinated biphenyls are now quite accessible through synthesis and recent studies have begun to obtain these types of biological data.

Studies using a group of five symmetrical hexachlorobiphenyl isomers in chicks (McKinney et al., in press) and in mice (Biocca,

MONS 056226

1975) indicated that distinct difference in toxicity are possible and these differences could be related to structure. The isomers with chlorine substituents in the 4,4' positions appeared to accumulate more rapidly in adipose tissue, showed increased activity in the liver and greater overall toxicity (McKinney, 1975; McKinney et al., in press). Comparison of two pentachlorbiphenyls in laying chicks again indicated the one isomer with 4,4'-substitution to have greater toxic effects (Ax and Hansen, 1975; Ax et al., 1976). Structure-activity relationships, particularly for biphenyls known to be major components of commercial PCB mixtures, could be useful in assessing the potential hazard of these compounds.

A major part of determining human exposure to PCB residues in foods is evaluating the adequacy of the analytical methodology used and the significance of the data obtained. The methods applicable to PCB determination are complex and to be judged adequate should give acceptably reproducible results in the hands of experienced analysts. The degree of success of inter-laboratory collaborative studies helps measure the adequacy of a method.

PCB residues are multicomponent mixtures. Many common chlorinated pesticides are extracted from samples along with the PCBs. Procedures for separation of PCB residues from interfering pesticides therfore bec ..e important as a prerequisite to quantitation. Several different quantitation techniques have been used (Hutzinger et al., 1974). Comparison of PCB residue data is difficult where standardization of the quantitation procedure is lacking.

MONS 056227

It has been shown that recovery of PCB mixtures purposely added to food samples, using commonly applied analytical methodology, varies with the average level of chlorination. Recoveries tend to decrease with increasing chlorine level (Stalling et al., 1972).

Analytical procedures for determination of PCB and PBB (the latter is predominantly a hexabromobiphenyl) residues are essentially similar. They differ somewhat in cleanup of the extract and in the use of a higher column temperature for gas chromatographic determination of the PBB residue. The limit of quantitation for PCBs is generally 0.2 ppm for individual foods and about 0.05 ppm for Total Diet composites using Food and Drug Administration methodology. For PBB residues, the limit of quantitation is approximately 0.05 ppm in fats and 0.01 ppm in non-fatty foods. Interlaboratory studies of analytical methods for PBB determination have not been reported.

The chlorinated dibenzofurans (Cl-DBFs) have become a focus of concern as the class of contaminant compounds in commercial PCBs most likely to contribute significantly to the toxicity of the PCB mixture. This concern presumably stems mainly from the demonstrated toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and the similarity in structure between dibenzo-p-dioxin and dibenzofuran (NIEHS Conference, 1973; also see Appendix of Chemistry Report) and from the little toxicity data that has been published for Cl-DBFs (Bauer et al., 1961). Recent data indicated

MONS 056228

the toxicity of 2,3,7,8-tetrachlorodibenzofuran approaches that of the analogous dibenzo-p-dioxin at least in chicks and in guinea pigs (Moore et al., 1976). If the chlorinated dibenzo-p-dioxins can serve as an example, chlorinated dibenzofuran toxicity can be expected to vary with number and position of chlorine atoms on the parent ring system. Relationships between structure and toxicity will emerge as more Cl-DBFs are synthesized and tested for toxicity.

A range of Cl-DBFs (from dichloro through hexachloro) have been reported in various Aroclors (Roach and Pomerantz, 1974b; Bowes et al., 1975a) and two specific compounds, the 2,3,7,8-tetrachloro- and 2,3,4,7,8-pentachloro-dibenzofurans, have been identified (Bowes et al., 1975b). Quantitation of Cl-DBF contaminants in PCB mixtures, a difficult procedure, suggests total Cl-DBF levels in the low parts per million range (Nagayama et al., 1975; Bowes et al., 1975a). The presence of Cl-DBF in a synthesized, individual, symmetrical chlorinated biphenyl has been reported (Moron et al., 1973). Chemical analysis of these individual chlorobiphenyls may be necessary prior to their use in toxicological and other biological studies.

There have been no published positive findings of Cl-DBFs in environmental samples or in foods other than rice oil. The finding of Cl-DBFs in such samples would not necessarily implicate commercial PCBs as the source of these contaminants since Cl-DBFs (generally the higher chlorine levels) have been reported in other

MONS 056229

industrial chemicals (Firestone et al., 1972; Schwetz et al., 1974; Villanueva et al., 1974). It is also possible that Cl-DBFs may be formed or altered in the environment.

METABOLISM AND BIOCHEMICAL TOXICITY

The effect of PCBs and PBB on the hepatic mixed-function oxidase (MFO) enzymes has been the most thoroughly studied of any biochemical parameter that they are known to alter. On a molar basis, the PBBs are approximately five-fold more potent than the PCBs in inducing increased levels of the MFO enzymes (Farber and Baker, 1974). Among the PCBs it appears as if their potency increases with increasing chlorination and chlorine substitution in the para > ortho > meta positions respectively (Echobichon, 1975). However, this induction of the MFO enzymes is not unique to these compounds and the induction observed is well within the range observed with many other xenobiotics. The PCBs are somewhat unique as MFO inducers in that they induce the formation of both Type I and Type II P-450 (Alvares et al., 1973), but this induction may have been due to the fact that the commercial formulation used in the study was a mixture of twenty or more PCBs which were metabolized to an even greater number of metabolites. In addition, many, if not most commercial PCB formulation contain trace amounts of chlorinated dibenzofurane (Araki, 1974). These compounds may be up to 170 times more potent as MFO inducers than the PCBs. The chlorinated dibenzofuran concentration of American

MONS 056230

PCB formulations is usually quite low when they are produced, however, the effect of long-term is unknown. In any case, since induction of the MFO enzymes may result in increased hormone metabolism or carcinogen activation, exposure to the PCBs and PBBs should be limited on that basis alone.

PCBs and PBBs administered at relatively high concentrations, usually 50 ppm or higher in the diet, to laboratory animals have been shown to cause prophyria (Goldstein et al., 1975), disfunction of the thyroid (Bastomsky, 1974), inhibition of various enzymes (Pardini, 1971), changes in the liver to body weight ratios (Garthoff et al., 1975) various disorders of the liver (Kimbrough et al., 1975), and to alter the level or utilization of corticosteroids (Wasserman et al., 1973) and vitamins A, D and E (Cecil et al., 1973; Wong et al., 1974; Combs et al., 1975). Certain of the less chlorinated PCBs have also been shown to have a mild estrogenic effect when tested in the immature rat and mouse (Kihlstrom, 1973; Nelson, 1974). However, most of these parameters have not been studied in sensitive species or demonstrated at low exposure levels in laboratory animals.

The available data imply that the PCBs and PBBs containing six or fewer halogen atoms are readily absorbed from the gut of higher animals. The available data also imply that the PCBs are not excreted to an appreciable extent prior to metabolism to more poler compounds, and that long term PCB storage is in the skin and adipose tissue (Matthews and Anderson, 1975a). Studies of PBB

MONS 056231

metabolism are not yet available. Since tissue samples from birds and mammals with known exposures to PCBs usually contain only those PCB# with five or more chlorine atoms, it is assumed that the less chlorinated PCBs have been metabolized and excreted. On the other hand there is little evidence that fish can metabolize any PCB and an analysis of fish tissue usually shows a PCB pattern very similar to that to which the fish were exposed (Stalling and Mayer, 1972).

Laboratory studies have demonstrated that the rate of PCB metabolism and thus excretion is approximately inversely proportional to the degree of PCB chlorination so long as there are two adjacent unsubstituted carbon atoms on the biphenyl molecule. When two adjacent unsubstituted carbon atoms are not present the biological half-life of the given PCB may be a matter of years and accumulation of high tissue concentrations with continued exposure is inevitable (Matthews and Anderson, 1975a). It should be noted that the major constituent of Firemaster BP-6 does not have two adjacent unsubstituted carbon atoms and that the corresponding PCB has been shown to have an extremely long half-life in the laboratory rat and probably the human population as well.

It should also be noted that metabolism can also result in further complications of the PCB problem because those PCBs which are most readily metabolized and excreted are those which are most likely to be metabolized via arene oxide intermediates. And where as the reactivity of arene oxides varies greatly and often

MONS 056232

ultimately determines the toxicity, mutagenicity or carcinogenicity of the parent compound (Jerina and Daly, 1974), the PCBs offer such a range of degree and position of substitution that it would not be unlikely to find that one or more of these PCB metabolites would have the proper stability to be a mutagen or a carcinogen. Thus a move from the more highly chlorinated PCB formulations to the less chlorinated ones may help solve the long-term residue problem only to intensify other problems.

ANIMAL TOXICOLOGY

Gleaning the information that is now available on animal toxicology makes it obvious that different commercial mixtures of PCBs elecit different toxic responses in animals, and that different animal species vary in their susceptibility to the toxic effects of PCBs. Reproduction is severely affected in mink at a dietary level of 5 ppm Aroclor 1254, and a slight effect is still noted at a dietary level of 1 ppm (Ringer et al., 1972). In rhesus monkeys reproduction was reduced at a dietary level of 2.5 ppm of Aroclor 1248 (Allen 1975). In rats a dietary level of 20 ppm Aroclor 1254 depressed reproduction while in the same rat strain (Sherman), in a study conducted simultaneously in the same laboratory, a dietary level of 500 ppm Aroclor 1260 was necessary to reduce reproduction (Linder et al., 1974). In comparative studies done with European products, Phenoclor DB6 and Clophen A60, and the American product, Aroclor 1260, the

MONS 056233

European products were more toxic to chickens than the American product (Vos & Koeman, 1970). The difference in toxicity in this study was attributed to contamination of the European products with chlorinated dibenzofurans and perhaps chlorinated naphthalenes. However, if the differences in the effect on reproduction by the different Aroclors are compared, the contamination with chlorinated dibenzofurans may not be the decisive factor.

Hepatic porphyria has been produced in a number of species, namely the chicken, rabbit, Japanese quail and rat, by a number of commercial mixtures, such as Clophen A60, Phenoclor DP6, Aroclor 1016, 1242, 1254, and 1260 (Vos and Koeman, 1970, Vos and Beems, 1971, Iverson et al., 1975, Goldstein et al., 1974, 1975). It (hepatic porphyria) has not been reported in the monkey, mink or human (see Yusho) mammalian species quite susceptible to the toxic effects of PCBs in other ways. Aroclor 1254 and Aroclor 1242 produced hepatic porphyria in the female rat at doses lower than Aroclor 1016. Again, different commercial mixtures produce this toxic effect at different dosage levels. The hepatic porphyria occurs concomitantly with an increase in ALA synthetase in the liver. Mixed function oxidases are also induced in the liver and comparative studies with PCB isomers have suggested that if the 4,4' positions on the biphenyl ring are occupied by chlorine atoms the effect is most pronounced (Ecobichon and Comeau, 1975).

The liver is the primary target organ for PCBs in the rat. Early changes include hepatomegaly with a concomitant increase

MONS 056234

in smooth endoplasmic reticulum, lipid accumulation at higher dietary levels (20 ppm for Aroclor 1254 and 1260) and ultra-structural changes such as atypical mitochondria and the formation [illegible] consist of [illegible] arranged membranes surrounding lipid vacuoles [illegible] noted with either higher [illegible] (Kimbrough, 1974). These changes are not as pronounced with Aroclor 1242 or 1016. Similar changes have been reported in the primate (Allen, 1975).

In the mouse liver tumors have been produced with Aroclor 1254 (Kimbrough and Linder, 1974) and Kanechlor 500 (Ito et al., 1973) and in the rat with Kanechlor 400 (Kimura and Baba, 1973) [illegible] have also been produced with Aroclor 1242, [illegible] 1260 [illegible] in a separate study. In one study with Aroclor 1260, some of the tumors were classified as [illegible] (Kimbrough et al., 1975). The dietary level [illegible] 100 ppm [illegible] high level [illegible] compared [illegible] but [illegible] polluted [illegible] emphasized [illegible] and [illegible] (2.5 ppm in the monkey [illegible]) PCB mixtures do not represent a no effect level. It is presently not known what the no effect levels are. Other uncertainties

MONS 056235

contribute to these problems. The Yusho oil which caused a poisoning outbreak in Japan was not only contaminated with PCBs but also with high levels of chlorinated dibenzofurans.

In the primate, in addition to the effect on the liver, the gastric mucosa (Allen and Borback, 1973), the skin, and the Meibomian glands are also affected at comparatively low dietary levels and the bone marrow is depressed (Allen 1975) while the gastric mucosa of the rat is only affected at exceedingly high doses (Kimbrough, 1974). Whether the dog also shows an affect on the gastric mucosa needs clarification. In the rabbit, atrophy of the thymus is a toxic manifestation in addition to liver pathology (Vos and Beems, 1971). Fluid accumulation occurs in primates, chickens, and finches. The lymphatic system is also affected in minks.

The few studies conducted with some hexachlorobiphenyl isomers demonstrated that the 3,4,5,3',4',5' hexachlorobiphenyl was the most toxic while 2,3,6,2',3',6' hexachlorobiphenyl was the least toxic isomer (Biocca et al., 1975). Penta-, hexa- and heptachlorobiphenyls are preferentially retained in mammalian adipose tissue for extremely long periods of time (Kimbrough, 1975) (at fairly high concentrations in the rat for a recovery period of 16 months). Whether this has an influence on the toxicity of PCB mixtures has not been determined.

The toxic effects of the contaminants of PCBs have not been extensively studied. While it is assumed that chlorinated

MONS 056236

naphthalenes are toxic within the same dosage range as the chorinated biphenyls, the chlorinated dibenzofurans probably have a greater toxicity. It is assumed that 2,3,7,8 tetrachlorodibenzofuran is the most toxic of this group of compounds. This compound also shows marked species variation. While the single oral LD_{50} in guinea pigs is between 5 and 10 ug/kg bodyweight, 1000 ug/kg TCDF given orally had no affect on rats. Mice are equally insensitive to the toxic effects of TCDF (Moore et al., 1976).

For the brominated biphenyls limited toxicity data are only available on mixtures containing predominately hexa- and octabromobiphenyl. Both mixtures differ sufficiently in isomeric composition so that their toxic effects may quantitatively be quite different and also different from the PCBs. Again the problem of toxic contaminants has not been resolved. If toxic effects are similar to PCBs then, at least in some species such as mink and monkey, longterm low level exposure should result in measureable toxicity. Additional animal studies are needed to resolve some of these problems. Poor metabolism and excretion of the brominated biphenyls may lead to long retention of these compounds predominantly in adipose tissue with accumulation to very high levels on continued exposure. Whether this would lead to sufficient recirculation of the chemicals to cause toxic effects on target organs in presently not known.

MONS 056237

HUMAN EXPOSURE

Several reports (Jelinek & Corneliussen, 1975; Humphrey, 1976; Kutz and Yang, 1975; Dennis, 1975; Kleinhert, 1975; Hesse, 1975; Yobs, 1972; Kutz and Strasamman, 1975) provide evidence that would indicate that a substantial proportion of the population of the United States has been exposed to PCB's. Minimal human exposure of the population to PCB's has occurred from food, air and water, while significant human exposure appears to be limited to sports fishermen consuming fresh water fish from contaminated streams and lakes, and to occupational exposure in industrial workers.

Jelinek & Corneliussen (1975), in reviewing data from the FDA Total Diet Study (1971-1975) report that all food classes of the total diet have declined to no PCB occurrences except in these meat-fish-poultry composites. About 40 percent of these composites continue to contain detectable residues of PCB's, although only traces have been detected in the latter years. The fact that levels in these composites have declined to only traces further support the inference that the meat, poultry and eggs no longer contain detectable PCB's and that the low level findings are probably due to the fish in these composites. This would imply that the PCB levels in the diet may have "bottomed out" and may remain static until such time as there is a change in the PCB residues in fish.

MONS 056238

Data compiled from studies sponsored by the National Marine Fisheries Service (NMFS) also support the continuing presence of PCB residues in fish. A compilation of PCB data, representing the results of all the measurements known to NMFS on PCB's in fish used in the U.S. diet, indicates several important points: (1) while at one time or another, some PCB measurements have been made on many fish, there is an inadequacy of information on PCB residues in the most important fish items in the fish diet; (2) sampling and analysis have been sporadic and not designed to measure trends in human exposure; and (3) systematic surveys of neither the important items nor the species most likely to be contaminated have been undertaken.

However, these survey data do show that in general U.S. fish eaters include a wide variety of fish items in their diet and that some 93 percent of the U.S. population (197 million) consume fish, with the average annual consumption of fish being 15 pounds/year/fish eater.

At present, it is difficult to estimate all human exposure to PCB from eating fish, either from the population as a whole or subgroups at higher risk of consuming large quantities of fish with higher PCB residues. Fragmentary evidence from NMFS data suggests that the exposure of the population as a whole from PCB residues in ingested fish is probably well below 19 mcg/day/consumer, based on PCB levels which are estimated to be below 1 ppm and 19 g of fish consumed/day. This can be compared to

MONS 056239

the results of the FDA Total Diet Study where it has been estimated that the overall PCB daily intake is on the order of 5-10 mcg/day for the general population. The lower FDA estimate is based on the methodology of the Market Basket Survey where fish are purchased at the consumer level and would not be applicable to diets which include a high consumption of fish from certain areas with high PCB residues.

A recently completed study (Humphrey et al., 1976) has attempted to assess some of the consequences of human exposure to PCBs from the high consumption of fish from contaminated areas. The results of this study show that a group of sports fishermen consumed an average of 24-25 pounds of fish/person/year, with the highest individual exposure for a two year period reported as 180 pounds per year. PCB residues in cooked fish ranged from 0.36 to 5.38 ppm. Although there was a wide range of blood PCB levels for each quantity of fish consumed, there was a highly significant correlation between the reported quantity of Lake Michigan fish consumed and the concentration of PCB in the blood of study participants, with the higher reported fish consumption being associated with higher PCB blood levels. The blood values ranged from a low of 0.007 ppm in the control group (fish consumption less than 6 pounds/year) to a high of 0.366 ppm in the exposed group (fish consumption 24-25 pounds/year).

These investigators calculated that the amount of PCB ingested by the exposed group could average 46.5 mg/year and ranged from

MONS 056240

14.17 to 114.31 mg/year. While no systematic adverse health effects could be demonstrated in the exposed group when compared to controls, the investigators caution that any long-term chronic effects are unknown at the present time. Additionally, it can be concluded that exposure similar to those reported in this special group will continue and there is the likelihood that as sportsfishing becomes more popular, larger numbers of people may be exposed in a similar way.

Although human exposure to PCBs from air and water is probably minimal, there seems little question that such exposure does occur. Samples of ambient air collected in Florida, Mississippi and Colorado, show that PCBs were present at all locations. The average concentration at each of the three locations was approximately 100 nanograms per cubic meter of air. Studies of surface water from the major drainage basins of the United States report the widespread occurrence of PCBs in both surface water and bottom sediments. Mean residue levels of PCBs in the surface water ranged from 0.01 to 0.05 mcg/liter, with a maximum residue level of 20.0 mcg/liter.

In Wisconsin, effluents from cooling water in aluminum foundries contained PCBs ranging from 11.5 to 335 ppb. Effluents from paper mills ranged from 0.01 to 25 ppb. Analysis of snow melt water from Wisconsin cities showed PCB residue levels of 0.17 to 0.24 ppb, suggesting that fallout of PCBs from the air may be an important source of PCBs entering the waters of the state.

MONS 056241

In Michigan, testing of 900 samples of industrial effluents showed 22 percent had PCB residues greater than 0.5 mcg/liter, 8 percent greater than 1.0 mcg/liter, 6 percent greater than 10 mcg/liter and 2 percent greater than 100 mcg/liter. With sludge disposal taking place by incineration, landfill and crop or pasture application, the continuation of PCBs in the environment seems obvious.

Adverse human health effects resulting from PCB exposure have come primarily from studies of occupational exposure and from human exposure through the ingestion of contaminated rice oil in Japan.

Schwartz (1936) provided some of the earliest reports of adverse health effects due to occupational exposure in the U.S., in which he described skin lesions and symptoms of systematic poisoning among workers who were reported to have inhaled chlorodiphenyls. There have been numerous reports over the ensuing years describing cutaneous eruptions and of systematic manifestations as well, among marine electricians, machinists, capacitor and transformer manufacturing workers, and others occupationally exposed to PCBs. The skin lesions described by Schwartz in 1936 have come to be designated as "chloracne." Part of the chloracne lesion resembles adolescent acne, but is generally more severe and the lesion distribution is inconsistent with adolescent acne.

Hara (1969) and Hasegawa et al., (1973) have reported dermatologic ailments which include "brown chromodermatosis" of the

MONS 056242

dorsal joints of the hands and purple-like eruptions of the face and neck. However, Hasegawa et al., (1973) performed a health survey of workers in carbonless copy paper factories, two years after the use of PCB in such processes had ceased, and reported no dermal effects nor liver function, urine or blood test abnormalities. PCB blood levels were reported as 0.01 - 0.02 ppm.

Typical clinical findings in the human exposure to PCB which occured in Japan in 1968, and which resulted from the ingestion of rice oil contaminated with Kanechlor 400 included chloracne and increased pigmentation of the skin, increased eye discharge, transient visual disturbances, feeling of weakness, numbness in limbs and some disturbance in liver function. Adult Yusho patients had protracted clinical disease with a slow regression of symptoms and signs. In the dose-response epidemiologic study, the average cumulative intake of PCB's leading to overt symptoms was 2,000 mg, with the lowest dose leading to overt symptoms being 500 mg.

However, Kuratsune et al., (1975) have introduced a new factor into the Yusho incidence with the finding that the rice oil contained chlorinated dibenzofurans (Cl-DBF) at 5 ppm. Nagayama et al., (1975) reports that the toxicity of (Cl-DBF) is said to be from 200 to 500 times that of PCB. Whether or not the (Cl-DBF) contaminant is the crucial toxic substance producing the symptoms observed in the Yusho incident, or whether the exposure to the high levels of Kanechlor 400 produced the observed effects, or

MONS 056243

whether there was an inter-active process taking place is unknown.

The data necessary for determining reasonably accurate time and dose exposure to PBB in individuals in Michigan is either unavailable or non-existent. Attempts to secure accurate dietary intake with the PBB levels in food and the duration of consumption have been unsuccessful. It is hoped that data to be received from the Michigan Department of Public Health may provide some basis for crude estimates.

While there appears to be no evidence at the moment to indicate acute health effects from exposure to PBB, any chronic effects remain largely unknown. A large scale epidemiological study is expected to get underway shortly to identify all the farm family members from quarantined farms; a large group of study subjects secondarily exposed to PBB through the purchase of farm products on a regular basis from quarantined farms and a control group of individuals not exposed to PBB contamination. This study will continue efforts to identify any acute or chronic effects of PBB exposure through physical examination, biochemistry tests, and dietary histories. Efforts will continue to assess the original Firemaster BP-6 for the presence, if any, of chemical contaminants which might present human health hazards. In the meantime, a ten-fold safety factor for PBBs when compared to PCBs appears both reasonable and acceptable based on all currently available scientific data.

MONS 056244

Much work remains to be done concerning the toxicity of PCBs and PBBs and the association of these compounds with any demonstrable adverse human health effects. To accomplish some of these needs a list of recommendations follows.

MONS 056245

GENERAL RECOMMENDATIONS

More complete and additional metabolism studies with various individual PCBs and PBBs should be undertaken. Attention to the formation of possible toxic metabolites of these compounds should be considered, as well as detecting and quantifying any specific biochemical parameters which may be affected as a result of such exposures. Based on the above, lifetime feeding studies should be conducted with selected commercial PCBs and PBBs mixtures, as well as selected individual PCBs and PBBs.

Why some species seem to be more susceptible to the toxic effects of PCBs, and which are most closely related to man in their response should be determined. In addition, more experimental data is needed for the occupational PCBs settings; i.e., via the respiratory or dermal route. Additional toxicological evaluations should be made of fish and other foods which contain high levels of environmentally accumulated PCBs and other contaminants. Appropriate toxicological studies should be undertaken with PBBs in order to be able to make some predictions on possible human effects.

The quantitative evaluation of halogenated biphenyl exposure to man with respect to blood and body fat levels, and any possible health effects, needs further study. With respect to PCBs, this may be accomplished by identifying a population of individuals consuming large amounts of fish contaminated with high levels of

MONS 056246

PCBs. In addition, those exposed industrially should be included in these efforts. With respect to PBBs, appropriate studies should be initiated with Michigan farm families exposed through consumption of contaminated meat and dairy products.

Individual chemical components of PCB residues should be qualitatively identified and procedures for improving quantitation of these residues should be investigated. Commercial PCB (and PBB) mixtures need to be analyzed further to determine which chlorinated dibenzofurans (or, in the case of PCBs, whether any brominated dibenzofurans) are present as contaminants. Specific chlorinated dibenzofuran compounds must be synthesized for use in development of analytical procedures and to aid in identification of contaminants. Analytical procedures are needed to permit examination of foods for presence of chlorinated dibenzofurans. Toxicological studies should be carried out on chlorinated dibenzofurans and brominated dibenzofurans.

MONS 056247

DETAILED RECOMMENDATIONS

CHEMISTRY

Investigate procedures (such as Webb and McCall's, J. Chromatog. Sci. 11, 366-373 (1973)) for possible improvement of the quantitation step in the analysis of PCB residues.

Present procedures for analysis of PCBs yield a "PCB residue" that is commonly examined using electron capture GC. Individual components of this residue should be identified and it should be determined whether any of the electron capture GC peaks can be attributed to Cl-DBFs or Cl-naphthalenes.

Analyze commercial PCB mixture (e.g. Aroclors) to determine which Cl-DBFs are present as contaminants. Qualitative and where possible, quantitative studies should be carried out.

Synthesized, individual chlorobiphenyls should be examined for presence of chlorinated dibenzofurans before metabolism or toxicity studies are carried out with the biphenyl. (The importance of this is shown by the reported formation of 2,3,7,8-Cl_4 DBF as a side-product in the Ullmann coupling reaction used to synthesize the symmetrical 2,2',4,4',5,5'-hexachlorobiphenyl.)

Synthesize, purify and characterise by physical and chemical means a range of Cl-DBFs to be used for analytical procedure development as reference standards for confirmation purposes and for suitable toxicological studies.

MONS 056248

Develop a suitable procedure for analysis of edible fish for Cl-DBFs, emphasizing recovery and quantitation of 2,3,7,8-tetrachlorodibenzofuran.

Analyze a commercial mixture of brominated biphenyls (Firemaster BP-6) for brominated dibenzofuran content.

Analyze long-used heat exchange, transformer and capicitor fluids for chlorinated dibenzofurans.

Synthesize specific ^{14}C or tritium radiolabeled chlorinated biphenyls that may be needed for toxicity and metabolism studies.

Study the reported conversion of certain chlorinated biphenyls to Cl-DBFs under sunlight or sunlight-simulating conditions. If feasible, extend the work to additional Cl-biphenyls.

Simulating conditions applicable to heat exchange units and/or transformers, determine whether Cl-DBF content of PCB mixtures increases when the PCBs are heated and/or exposed to air.

Carry out laboratory studies, simulating environmental conditions, to determine alteration products from Cl-DBFs when irradiated or heated.

METABOLISM AND BIOCHEMICAL TOXICITY

Long-term feeding studies approaching life-time should be conducted with the less chlorinated PCB formulations. These

MONS 056249

studies should include at least two of the less chlorinated PCB formulations and a series of carefully selected individual PCBs.

Further and more complete metabolism studies should be done with the more highly chlorinated PCBs, for comparison with those done with PCBs having five or less chlorine atoms.

The effect of chlorine position on PCB metabolism and arene oxide formation should be further elucidated.

The formation of specific chlorinated dibenzofurans as possible PCB metabolites should be studied, their biochemical toxicity in mammalian species determined and the magnitude of their toxicity ascertained. These studies will also include those specific chlorinated dibenzofurans identified in new and used PCBs.

If possible, arene oxide metabolites of several PCBs should be synthesized and their toxicology investigated.

Within an isomeric series of PCBs, (e.g., tetra or hexa), metabolism of the more toxic members should be compared to those which are less toxic.

Metabolism of individual PBBs, comparable to those for PCBs, should be studied. These data should be related to known toxicities.

MONS 056250

The toxicology of known PCB metabolites should be studied.

Porphyrin excretion by the people and animals exposed in the Michigan PBB incident should be checked.

People who are suspected to have received high exposures of PCBs or PBBs should be checked for any changes in enzyme levels--e.g., protein-bound iodine, antipyrene metabolism, serum cholesterol, etc.

The adsorption, distribution and excretion studies of halogenated dibenzofurans and PBBs should be studied.

Further studies of possible PCB metabolism by fish.

Study the degree of chlorination and length of exposure on the estrogenic effects and reproductive organs with particular emphasis on the lower chlorinated PCBs (e.g., 1016).

ANIMAL TOXICOLOGY

Inhalation studies with "heated" PCB mixtures simulating occupational exposure and dermal toxicity studies should be conducted.

Studies as to why some species seem to be more susceptible to the toxic effects of PCBs than others and of these species, which are most closely related in their response to humans should be undertaken.

MONS 056251

Subacute and chronic toxicity studies should be conducted with hexabromobiphenyl (Firemaster FF-1) in order to be able to make some predictions on human PBB toxicity.

The toxicity of fish containing high levels of PCBs which have been accumulated by environmental exposure should be evaluated. It should be established whether and which toxic impurities are present in used PCBs, such as transformer oil and capacitor fluid.

Long-term feeding studies, approaching life-time, should be conducted with laboratory animals and selected commercial mixtures of PCBs and PBBs as well as selected individual PCBs and PBBs.

Further and more complete metabolism studies should be done with individual PCBs and PBBs. The formation of toxic metabolites of these compounds should be considered and carefully investigated.

Efforts to detect and quantitate specific biochemical mechanisms which are affected or altered by exposure to PCBs or PBBs should be continued.

HUMAN EXPOSURE

Polychlorinated biphenyls

Recommend that a population of fish eaters be identified who are consuming substantial amounts of fresh water fish with high levels of PCBs. This study population should be followed prospectively with adequate dietary histories, blood and body fat levels

MONS 056252

of PCBs, a health questionnaire, liver function tests and other biochemical studies.

Recommend a similar study be carried out in an industrial population exposed to PCBs.

Systematic studies to identify sub-groups of the total U.S. population who consume high levels of fish with more accurate measures of PCB exposure.

Polybrominated biphenyls

Recommend that a large scale epidemiological study be carried out to (1) identify all farm family members from quarantined farms in Michigan, (2) identify those individuals with secondary exposure to PBB contamination through the purchase of dairy products from quarantined farms on a regular basis, and (3) a suitable control group of non-exposed farm families. These groups should be followed to assess any short and long term adverse health effects related to PBB exposure.

Subgroups of these farm families from quarantined farms should be studied by:

1. Repeat PBB blood levels.
2. Matched blood-body fat PBB levels.
3. Pregnant female-infant studies.
4. A battery of biochemical studies.

MONS 056253

Ancillary Studies

Toxicology studies in non-human primates and rodents with hexabrominated biphenyl and any brominated contaminants of hexabrominated biphenyls.

Toxicology studies in several animal species (mice, rats, dogs, non-human primates using hexabrominated biphenyl and several PCBs to determine species differences in response to the effects of these compounds.

Toxicology studies feeding hexabrominated biphenyls and selected PCBs singly and in combination to determine if there are different or additive effects when fed in combination.

MONS 056254

CHEMISTRY OF PCBs AND PBBs

I. Introduction

A recent book by Hutzinger et al. (1974) reviews the chemistry of chlorinated biphenyls through the end of 1973. This wide-ranging review also includes discussions of metabolism and determination of chlorinated biphenyls.

The present report reviews areas of chlorobiphenyl and bromobiphenyl chemistry held to be pertinent to the Subcommittee's concern for possible health effects of these types of compounds. No attempt at complete coverage of these subject areas is claimed. Findings reported since 1973 are particularly stressed.

Non-metabolic alteration of the halogenated biphenyls is considered in terms of agents most available to induce chemical change in the environment. Expected differences between chlorinated biphenyls and brominated biphenyls in their environmental chemistry are commented on.

It is possible that certain of the chlorinated biphenyl compounds will be shown to have relatively greater potential hazard for health. Therefore, occurrence and fate in the environment and metabolism and toxic effects in animals are related to chemical structure of the biphenyls.

For the general public, food is presumed to be the major

MONS 056255

source of chlorinated biphenyl compounds. The important concepts related to analytical methodology for PCB (and PBB) residues, particularly in foods, are reviewed. Alternative means of quantitation of these residues and problems in the quantitation techniques are discussed.

The pertinent aspects of chlorinated dibenzofurans, as known contaminants in commercial PCBs and as potentially significant environmental contaminants, are reviewed.

In reviewing the chemistry related to health effects of the PCBs and PBBs, it must be remembered that environmental contamination by these two commercial chemical mixtures is vastly different in magnitude. The former have been steadily released into the environment, in many countries, presumably over decades, and are now found to be a pervasive, world-wide contaminant. The number of chlorinated biphenyls reaching the environment probably number nearly 100 different compounds. The PBBs, encompassing a small number of chemical structures to begin with, are of concern due to a single, fairly recent contamination incident, apparently limited to the State of Michigan.

II. Chemistry of Chlorinated Biphenyls

A. Synthesis and Analysis of Commercial Mixtures

Commercial preparation of mixtures of chlorinated biphenyls by reaction of biphenyl with chlorine has been described by Hubbard (1964). A more recent discussion of this subject,

MONS 056256

including the newer preparation designated as Aroclor 1016, is now available (Mieure et al., 1976). Analysis of the chlorinated biphenyl content of various American commercial PCB mixtures (Aroclor) has been reviewed by Hutzinger et al. (1974). An estimated 40 to 60 different chlorinated biphenyl compounds are present in each of the higher chlorinated commercial mixture. (There are 209 possible compounds obtainable by substituting chlorine for hydrogen on from one to ten different positions on the biphenyl ring system; see Appendix.) PCB commercial mixtures produced in the U.S. and elsewhere have been shown to contain classes of compounds other than the chlorinated biphenyls: chlorinated napthalenes and chlorinated dibenzofurans (Cl-DBFs), for example (Vos et al., 1970; Roach and Pomerantz, 1974 a,b; Bowes et al., 1975a). The possibility that naphthalene and dibenzofuran contaminate the technical biphenyl feedstock used in preparation of the commercial PCB mixtures has not been excluded.

Available toxicity information indicates that, of the identified types of contaminants in commercial PCB mixtures, Cl-DBFs pose the greatest potential hazard (See Section II.E on Chlorinated Dibenzofurans). As a result, the Cl-DBFs have become the focus of studies on contaminants in these mixtures. No report has appeared of an attempted complete content analysis of the trace impurities in a commercial PCB mixture.

MONS 056257

B. Non-metabolic Alteration of Chlorinated Biphenyls

Chlorinated biphenyls, as is typical of aryl chlorides generally, are quite stable to chemical alteration. Consideration of possible non-metabolic alteration routes for these compounds in the environment suggests air oxidation, aqueous hydrolysis, and photoalteration in sunlight as potential reactions to be investigated. (Thermal decomposition would be of concern in connection with incineration conditions meant to destroy waste chlorinated biphenyls). Study of these reactions under the complex and variable sets of conditions existing in the environment is difficult. Chemical reactivity of chlorinated biphenyls has therefore been studied almost entirely under the more controlled conditions of the laboratory.

Oxidation

PCBs are fairly stable to oxidation under moderate conditions. PCBs are stable under conditions which easily oxidize DDE, and can be separated from DDE by oxidizing DDE to the more polar dichlorobenzophenone prior to column chromatographic separation (Trotter, 1975). To effect the oxidation of DDE, the PCB-DDE solution is refluxed in 2.5% chromic acid-acetic acid on a steam bath. Some of the lower chlorinated biphenyls, however, are not recovered after the oxidation. It is reported (Weingarten, 1961) that mono-, di-, and trichlorobiphenyls are oxidized in 7.5%

MONS 056258

chromic acid-acetic acid to their respective (chloro-) benzoic acids. With vigorous oxidizing conditions in the environment, some PCB oxidation, especially of the lower chlorinated biphenyls may occur. It is difficult, however, to assess the total extent of possible environmental oxidation of PCBs.

Hydrolysis and Alcoholysis

PCBs are fairly stable to hydrolysis under moderate conditions. When refluxed with 2% KOH in ethanol, PCBs are stable (Trotter, 1975; Young and Burke, 1972). A halogen on a chlorobiphenyl molecule is not easily displaced in a nucleophilic substitution reaction. Under vigorous conditions the 4 and the 4,4' positions of decachlorobiphenyl are found to be the most susceptible to chlorine displacement by hydroxide and methoxide ions. Decachlorobiphenyl can be hydrolyzed to octachloro-4,4'-biphenylol when treated with aqueous alkali at high temperatures in an autoclave (Smith, 1948; Societe d'Electro Chimie, 1962). Heating 2,5-dichlorobiphenyl with sodium methoxide produces 2-chloro-5-biphenylol (deCrauw, 1931). Decachlorobiphenyl when treated with sodium methoxide in pyridine yields the 4-methoxy- and 4,4'-dimethoxy-chlorobiphenyl (Binna and Suschitzky, 1971). It is probable that the non-metabolic hydrolysis or alcoholysis of PCBs in the environment is limited.

Photochemistry

Exact environmental conditions for the photochemistry of

MONS 056259

environmental contaminants, including PCBs, are sometimes difficult to simulate in the laboratory. Limited understanding exists concerning the parallel of laboratory photoreactions and reaction rates under non-environmental conditions and possible environmental photolyses. The exact environmental conditions under which residues may photoreact can be diverse and difficult to evaluate. Unknown or discounted factors in the environment, such as possible sensiters or quenchers, may be important. The frequency (energy) and intensity of a laboratory photolysis light may not be comparable with environmental conditions. 3000 Å probably represents the practical lower limit of the ultraviolet (UV) portion of sunlight (Crosby, 1969). The residue must be exposed to sufficient light to react appreciably. It is necessary to consider the physical state of the residue exposed to light in the environment. The residue may exist as a solid or liquid (e.g., a film), a solution or a vapor. In photolyzing solutions the solvent can have an important effect. Photoreactions which proceed in one solvent may not proceed or may proceed at a different rate in another solvent. A muddy river containing a residue may yield different results compared to a laboratory photolysis in hexane. Laboratory photolyses under a variety of conditions which simulate most, if not all, of the environmental conditions of photolysis could be extremely significant and relevant. In the absence of these, we must rely on the questionable extrapolation of laboratory photolyses under non-environmental conditions.

MONS 056260

The photochemistry of PCBs has been described by Hutzinger, et al.,(1972; 1974). In contrast to oxidation and hydrolysis, PCBs are fairly easily photoreacted under certain laboratory conditions. PCB photochemistry has been studied in various solutions, as a thin film, and as a vapor. In hydrocarbon solvents progressive reductive dechlorination of the PCB is the predominant photochemical reaction (Hutzinger et al., 1974). Irradiation of solutions of chlorobiphenyls (Hutzinger et al., 1972) and trapped gas liquid chromatographic (GLC) effluents (Hannan, et al.,1973) have shown that PCBs with higher chlorine content dechlorinate more readily. 0.1% hexane solutions of 2,2',5,5'-tetrachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl were each irradiated with 3100 Å light in a Rayonet reactor (Hutzinger et al., 1972). After 24 hours of irradiation, 33% of the 2,2',5,5'-tetrachlorobiphenyl was unreacted while < 1% of the 2,2'4,4',5,5'-hexachlorobiphenyl remained. In dechlorinating PCBs by photoexcitation, ortho chlorines preferentially cleave (Ruzo et al., 1974). With PCBs containing only meta and para chlorines, meta chlorines are lost preferentially. The rate of dechlorination is faster in alcohol solvents, such as methanol (Hustert and Korte, 1972) than in hydrocarbon solvents. Irradiation of 2,2',4,4',5,5'-hexachlorobiphenyl in methanol yields, in addition to reductive dechlorination, ring methoxylation (Ruzo et al., 1974). Photochemical studies of PCBs in aqueous solutions may offer a significant relevance to environmental PCB photochemistry. A 0.4% Aroclor 1254 suspension in water-dioxane (7+3) in the presence of sodium bicarbonate with

MONS 056261

air bubbling through the mixture was irradiated with 3100 Å light. Two thin layer chromatographic fractions of the Aroclor 1254 after irradiation corresponding to the "addition of water to chlorobiphenyls" and a "carboxy fraction" were found (Hutzinger et al., 1972). Presumably PCBs formed by reductive dechlorination were also produced. Black light irradiation of Aroclor 1254 as a thin layer film in the presence of water yields a "carboxy fraction" and a fraction whose mass spectrum indicates hydroxychlorobiphenyls (Hutzinger et al., 1972). Sunlight irradiation of certain chlorobiphenyls as a thin film without the presence of water gives reductive dechlorinated products (Hutzinger et al., 1974). Chlorinated terphenyls and quaterphenyls are also produced by the black light and sunlight irradiation of certain chlorobiphenyls as a thin film (Hutzinger et al., 1974). Vapor phase photolysis of PCBs may be extremely relevant especially for the more volatile components of Aroclors with low chlorine content. Sunlamp irradiation of the vapor phase of a refluxing suspension of 2 2',5,5'-tetrachlorobiphenyl and water yields "carboxy" products (Hutzinger et al., 1972).

These laboratory photoreactions represent chemical conversions which may proceed in the environment. The photoreaction rates under diverse environmental conditions and the extent of PCB degradation or reaction in the environment are extremely difficult to assess.

MONS 056262

The reported low-yield conversion of certain chlorinated biphenyls to Cl-DBF is discussed under Chlorinated Dibenzofuran, Section II.E.

C. Chemical Structure Related to Occurrence, Fate and Effects of Chlorinated Biphenyls

The physical and chemical properties of chlorinated biphenyl compounds and commercial mixtures of PCBs vary greatly depending on the degree and position of chlorine substitution on the biphenyl ring system. Of particular importance to their environmental occurrence and fate are the properties of volatility, water solubility, bioaccumulation, biodegradability and photostability. Volatility, water solubility and bioaccumulation are of more importance as mechanisms of introduction into and transport within the environment. Biodegradation and photodegradation are more important as mechanisms of removal.

There is data (Mieure et al., 1975) which indicates that the higher chlorinated biphenyls are less volatile and less water soluble than the lower chlorinated biphenyls. These two factors would tend to enhance the ratio of lower to higher chlorinated biphenyls in the environment. There is no clear data to assess the involvement of chlorine position in volatility and water solubility, but is likely to be of less importance. Therefore, the lower chlorinated biphenyls are more likely to volatilize into and be selectively transported in the aqueous environment.

MONS 056263

The relationships between structure and bioaccumulation factors for PCB isomers are only beginning to be assessed (McKinney, 1975; Sugiura et al, 1975) with the availability of purified specific isomers for study. However, it appears (Zitko et al., 1972; Veith, 1975) that the peak patterns from gas chromatography found in environmental samples representing the biosphere most closely resemble the higher chlorinated commercial mixture, Aroclor 1254. This suggests a selective accumulation of the mores highly chlorinated components of the commercial mixtures in biological material. This also indicates that the higher chlorinated biphenyls are able to find their way into the environment in spite of their poorer volatility and watar solubility. Pharmacokinetic studies (Matthews, 1975) with selected radiolabeled chlorinated biphenyl compounds have confirmed the trend toward increasing biological half-life with increasing chlorine number. However, these findings may be the result of both higher accumulation rates and lower elimination rates for the higher chlorinated biphenyls. It may be of interest to note that fish from lower and intermediate levels of the food web have been found (Zitko et al., 1972) to contain lower amounts of hexa and higher amounts of tetra and penta than higher trophic level white and silky sharks and aquatic birds This suggests that selectivity in bioaccumulation is also a function of biospecies.

Again the effects of varying chlorine position are less

MONS 056264

clearly understood, and the best assessment of this comes from study of two or more members of an isomeric series of the chlorinated biphenyls. Recent work with five symmetrical hexachlorobiphenyl isomers in chicks (McKinney et al., in press; Goldstein et al., in press) and subsequently in mice (Biocca, 1975) has demonstrated that separate and distinct differences in isomer toxicity are possible and that the differences are related to chemical structure via effects of varying chlorine substitution on compound lipophilicity and metabolism. The hexachloro isomers studied were 3,4,5,3',4',5'-; 2,4,6,2',4',6'-; 2,3,4,2',3',4'-; 2,4,5,2',4',5'-; 2,3,6 2',3',6'- and 2,3,5 2', 3',5'-. Those hexa-isomers with 4,4'-substitution appear to be more rapidly accumulated and may be more slowly metabolized, i.e., these isomers showed greater accumulation in adipose tissue and increased activity in the liver and overall greater toxicity. These differences are believed to be associated with differences in molecular polarizability and to be measureable by spectroscopic and chromatographic techniques.

Other workers (Ax et al., 1976; Ax and Hansen, 1975) have compared the toxicity of two pentachlorobiphenyls in laying chicks. The isomer with 4,4'-substitution (2,4,5,3',4') showed higher average embryonic mortality and teratogenicity in unhatched eggs but lower decreased fertility in the chick than 2,3,6,2',3' the isomer not chlorinated in the 4-position. Although it is difficult to assess the role of metabolism here, the data would suggest a

MONS 056265

more rapid accumulation of the 2,4,5,3'4'-isomer in the egg. Other workers (Bush et al., 1974) have already observed a correlation between PCB content of eggs and embryo mortality and teratogenicity.

Various other workers (Ecobichon and Comeau, 1975; Johnstone et al., 1974; Hill et al., 1974) studying a range of chlorinated biphenyls have generally observed the importance of degree of chlorination as well as position of chlorine substitution (especially 4,4'-substitution) in overall biological effectiveness. Although some of the compounds studied are unrealistic as components of commercial PCB mixtures, they have served as models to demonstrate the importance of chlorine number and position. Nevertheless, of 50 possible components of Aroclor 1254 identifed by Sissons and Welti (1971) as many as 19 (38%) could have 4,4'-substitution. It is of interest to note here that one of the possible persistent isomers found in Yusho patient tissue has been tentatively identified (Kuratsune, 1975) as the 2,3,4,5,3',4'-heptachlorobiphenyl. This isomer has been prepared (McKinney, personal communication) and its toxicty will be determined especially in relation to the highly toxic 3,4,5,3',4',5'-hexa isomer previously tested.

It has been only recently that hydroxylated metabolites of PCBs have been isolated and identified (Jansson et al., 1975) in environmental samples. The study of the metabolism of the commercial mixtures (deFreitas and Norstrom, 1974) themselves has

MONS 056266

received little attention for obvious reasons. Recently, the metabolism of purified chlorinated biphneyls, some radiolabeled for quantitation purposes, has been studied in a number of biological systems. Although there are a large number of publications in the literature on this subject, this report will concern itself with those studies which seem to support generalities in terms of the effects of varying chlorine number and position on metabolism with particular reference to mammalian systems and to realistic components of Aroclors.

The distribution and excretion of a series of four radiolabeled (^{14}C)chlorobiphenyls which have degrees of chlorination similar to and are themselves constituents of Aroclors 1221, 1242, 1254, and 1260 have been studied (Matthews, 1975) in the male rat. These studies have clearly shown an increasing biological half-life with increasing chlorine number for the series (4-; 4,4'-; 2,4,5,2',5'- and 2,4,5,2',4',5'-) which is believed to be related to the rate of metabolism and the ease of formation of the arene oxide intermediate. These compounds were also dosed at realistic exposure levels (0.06 to 6.0 mg/kg) which showed that little if any of the chlorobiphenyl is excreted in unchanged form. Most other metabolic studies (Hutzinger et al., 1974) with selected chlorobiphenyls have dealt with much higher dose levels (generally resulting in excretion of much unchanged biphenyl) and unlabeled compounds which are of little quantitative value in assessing the effects of chlorine degree and position.

MONS 056267

There has been no similar study with selected chlorinated biphenyls within an isomeric series to assess the effects of chlorine position on metabolism with the possible exception of an incomplete study (Hass et al., unpublished) on the identification of metabolites from the excreta of chicks (and mice) fed symmetrical hexachlorobiphenyl isomers. It is essential that this be done with symmetrical isomers (in order to simplify the probelm of interpretation) and preferably in two or more isomeric series (tetras and hexas).

The various metabolism studies have generally shown the occurrence of polar hydroxylated compounds as major components of the metabolite mixture. As the degree of chlorination increases, hydroxylation can be concurrent or concomitant with dechlorination (Hutzinger et al., 1974). There is also evidence for the formation of methyl ethers (Safe, 1975) and methylchlorobiphenyls (Hass et al., unpublished) as metabolites.

There is an increasing body of evidence (Safe et al., 1975) to support the arene oxide intermediate in hydroxylation, especially (Chen et al., Division of Nutrition and Disposition, in press) in cases where vicinal (1-2,-adjacent) unsubstituted carbons are found in the molecule. The corresponding dihydrodiol and/or catechol generally occur along with the phenolic metabolites.

Althought it has not been studied in mammalian systems, there is at least one report (Baxter et al., 1975) that studies of

MONS 056268

individual chlorinated biphenyls do not accurately predict the rates of metabolism of the same compound in simple mixtures. Therefore, the overall problem of metabolic degradation of the commercial mixtures may be complicated by the possibility that certain PCBs are potent enzyme inducers but poor substrates and vice versa with various degrees in between. This is further complicated by the fact that certain chlorinated biphenyls occur in optically active forms and only one enantiomer may be biologically active and undergo enzyme interactions. The existence of nine of the major, and ten of the minor, constituents of Aroclors 1242, 1254, and 1260 in optically active forms has been predicted (Kaiser, 1974).

D. Determination of PCB Residues

The determination of PCB residues has been reviewed in detail by the WHO Task Group report on "Environmental Health Criteria for Polychlorinated Biphenyls and Terphenyls" (WHO Task Group, 1975) and in "Chemistry of PCBs" (Hutzinger et al., 1974).

PCBs are lipophilic, quite similar in this respect to DDE, the metabolite of DDT. An analyses for PCB residues follow procedures the same as or similar to those used for multiple residues of organochlorine pesticides (WHO Task Group, 1975; Hutzinger et al., 1974; Food and Drug Administration, 1968-1975, Horwitz, 1975; Hearing Clerk, Dept. HEW, 1973). The individual steps that follow sampling are extraction of residues from the sample, isolation of residues from coextractives (cleanup),

MONS 056269

separation of PCBs from interfering chlorinated hydrocarbon pesticides, quantitation, and confirmation of identity. In general analytical methods capable of completely extracting residues of organochlorine pesticides from sample substrates and of quantitatively recovering the pesticides through subsequent cleanup procedures are also capable of achieving quantitative analysis for PCBs. The lipophilicity of chlorobiphenyls which increases with increasing chlorine content may influence their recovery through some analytical methods (Stalling et al., 1972). For example, recoveries through the frequently used cleanup step, partitioning of PCB from a petroleum ether or hexane solution of fat or oil to acetonitrile, exceed 95 percent for Aroclor 1242 but drop to 75-80 percent for Aroclor 1260 (Hearing Clerk, Dept. HEW, 1973). Overall ability of typical analytical methodology to recover PCBs added in vivo to food samples is shown by two interlaboratory studies conducted within the Food and Drug Administration (FDA) and one study conducted for the Association of Official Analytical Chemists (AOAC) (Food and Drug Administration, 1971; Burke, 1972; Food and Drug Administration, 1973; Sawyer, 1973). Using methodology described in the FDA Pesticide Analyticel Manual and in the Book of Official Methods of the AOAC, average recoveries and coefficients of variation for determination of Aroclors added as unknowns to different foods were: Aroclor 1254-fish, 74 $\pm$ 9 percent; Aroclor 1254-infant chicken, 89 $\pm$ 22 percent; Aroclor 1242-chicken fat, 101 $\pm$ 13 percent; Aroclor 1248-chicken fat, 96 $\pm$ 9

MONS 056270

percent;Aroclor 1254-fish, 75 ± 14 percent; Aroclor 1260-fish, 75 ± 15 percent. The three studies involving fish required separation of the PCBs from the DDT group before quantitation.

Gas chromatography with electron capture detection is the most widely used procedure for determination of PCB residues. Gas chromatographic columns with methyl silicone liquid phases are widely used under conditions that typically separate the Aroclors and PCB residues into about 15 peaks (Fishbein, 1972). The 10 percent DC-200 (or OV-101) column described in the FDA Pesticide Analytical Manual separates Aroclor 1254 into 14 peaks (Armour, 1972). These analytical columns do not give a highly defined representation of the Aroclor or PCB residue; several of the peaks in Aroclors result from mixtures of more than one chlorobiphenyl (Sissons and Welti, 1971; Stalling and Huckens, 1971; Webb and McCall, 1972; Webb and McCall, 1973). A column prepared from purified Apiezon L has been shown to separate a 54 percent chlorine PCB mixture into over 40 peaks (Jensen and Sundström, 1974). Separation of the PCB residue into three fractions by chromatography on charcoal prior to examination on the Apiezon L column has made it possible to characterize and quantitate nearly 60 technical PCB components. This method provides a way to get much needed, more detailed information on the compositon of PCB residues and might be used in special studies. The increased time and complexity of this approach would probably limit its application in regular monitoring analysis.

MONS 056271

The need to confirm residue identity and the procedures available are similar for PCBs and pesticides. The multipeak gas chromatographic pattern of a PCB residue may be very similar to that of a commercial Aroclor or, as in most biological samples, the residue peak pattern may be changed to varying degrees from that of a given Aroclor. The use of column chromatographic or chemical reaction procedures to separate PCBs from organochlorine pesticides increases the certainty of the gas chromatographic identification. Procedures readily available to the residue laboratory for confirming the identity of PCBs include: halogen specific gas chromatographic detectors (Hearing Clerk, Dept. HEW, 1973), stability of the residue peak pattern after refluxing the extract with alcoholic alkaline solution (Young and Burke, 1972), perchlorination of the residue to the decachlorobiphenyl derivative (Berg et al., 1971; Armour, 1973), and thin layer chromatography (Fehringer and Westfall, 1971; deVos and Paet, 1971). Mass spectrometry is not readily available to many laboratories conducting analyses for PCBs nor would the expense justify regular use in monitoring programs. However, the use of mass spectrometry is encouraged for residues and samples which are unusual or significant and for the occasional examination of a so-called routine sample.

Two especially critical considerations are associated with the determination of PCB residues: (1) their separation from potentially interfering organochlorine pesticides, particularly

MONS 056272

DDT, TDE, and DDE or the multicomponent chlordane or toxaphene, and (2) quantitative measurement of the multicomponent PCB residue which, in biological organisms, is usually changed in relative amounts of chlorobiphenyl components from commercial Aroclors or which may result from mixture of PCB from different sources. To achieve reliable residue results it is essential that the analyst correctly make critical judgements and interpretations in dealing with mixed residues of PCBs and organochlorine pesticides and in quantitation of the multicomponent PCB residue. There is no substitute for analyst experience in this analysis.

PCBs are separated from certain pesticides in the usual cleanup procedures, e.g., adsorption chromatography on Florisil or alumina or by gel permeation chromatography (Food and Drug Administration, 1968-1975; Stalling et al., 1972; Holden and Marsden, 1969). When DDT, TDE, DDE, chlordane, or toxaphene are present, ancillary procedures designed to separate these chemicals from PCBs must be used. The DDT analogs, particularly DDE, are the pesticide residues most frequently encountered in samples and DDE is the most difficult to separate from the PCBs. The effective electron capture gas chromatographic response for DDE is 20-30 times greater than for an equal weight of Aroclor 1254 and the response for DDT is only slightly less than that for DDE (Food and Drug Administration, 1968-1975). Without proper treatment these pesticides can readily interfere in the determination of PCBs. Some procedures utilizing column chromatography

MONS 056273

on alumina or Florisil separate DDT and TDE and intentionally collect DDE and PCBs in the same fraction (Holden and Marsden, 1969; Reynolds, 1969), which is analyzed. In these cases the analyst must exclude the DDE region of the gas chromatogram from the quantitative measurement of PCBs. Column chromatography on charcoal also has been proposed for separation of PCBs from DDE, DDT, and other organochlorine pesticides (Berg et al., 1971). Column chromatography on silicic acid is probably the most widely used procedure for separating PCBs from DDT and analogs (Food and Drug Administration, 1968-1975; Armour and Burke, 1970). The technique is fairly lengthy and difficult to reproduce, requiring empirical standardization in each laboratory (Sawyer, 1973; Masumoto, 1972; Edwards, 1974). The separation of DDE is probably not 100 percent effective but under ideal conditions practically all DDE can be separated; the usual inconsistency is for a portion of the DDE to be eluted from the column with the PCBs, requiring allowances to be made in PCB quantitation. The lower chlorinated PCBs present the greatest difficulty in separation from the DDT group by column chromatographic procedures. DDE can also be separated from PCBs by oxidation to the dichlorobenzophenone, followed by a chromatographic separation of this more polar derivative from PCBs. DDT and TDE may be dehydrochlorinated to their respective olefins and similarly separated along with DDE (Mulhern et al., 1971; Collins et al., 1972; Trotter, 1974). This technique has not received as much application as the chromatographic procedures, probably because pesticides and lower

MONS 056274

chlorinated biphenyls are destroyed or changed, preventing their determination. A variation on this approach used sodium dichromate plus a minute amount of sulfuric acid, rather than dehydrochlorination followed by oxidation with chromium trioxide in acetic acid. The dichromate reagent is reported to convert DDE quantitatively to the dichlorobensophenone without affecting DDT, TDE, or any of the chlorobiphenyls (Jensen and Sundström, 1974).

Quantitation of PCB residues is done in most laboratories by comparison of measurements made on the multicomponent electron capture gas chromatograms of the residue and a known quantity of reference material. The PCB residue in biological samples, as mentioned earlier, is very likely to be changed in the relative amounts of chlorobiphenyl components from any one Aroclor or the residue may be a mixture of PCBs from different sources (Food and Drug Administration, 1968-1975; Jensen and Sundström, 1974; Cook, 1972). The response of the electron capture detector varies with the number and location of chlorine atoms in the biphenyl molecule (Gregory, 1968; Zitko et al., 1971). With the electron capture system described in the FDA Pesticide Analytical Manual, the response/unit/weight increases about 6 fold from Aroclor 1242 to Aroclor 1260 (Hearing Clerk, Dept. HEW, 1973). The halogen specific microcoulometric and electrolytic conductivity detectors respond proportionally to the weight of chlorine present and in theory could provide a more accurate measurement of a PCB residue that is not exactly the

MONS 056275

same composition as the reference (Hearing Clerk, Dept. HEW, 1973). However, lower sensitivity, difficulty in maintaining optimum performance, and limited availability in residue laboratories has limited the use of these detectors. Unless the PCB residue and reference Aroclors are exactly the same in chlorobiphenyl composition, the gas chromatographic determination cannot be considered accurate; the greater the difference between residue and reference composition, the greater the deviation between determined and the true residue level (Hearing Clerk, Dept. HEW, 1973; Beezhoold and Stout, 1973). The most frequently used approaches to quantitation have been selected for the reference the Aroclor with the most similar gas chromatographic pattern to the residue and: (1) compared the response of a single peak in the residue with the response of the counterpart peak in the Aroclor reference; or (2) compared the total response for several peaks from the residue to the total response for the counterpart peaks in the reference; or (3) compared the total response for all peaks in the residue with the total response for all peaks in the reference; or (4) in a greater effort to duplicate the residue peak pattern, prepared a reference made up of one or more Aroclors to simulate the gas chromatographic pattern of the residue, and compared the total response for all peaks of the residue to the total response for all peaks in the reference (Sawyer, 1973; Beezhold and Stout, 1973). Response has been measured in terms of both peak height and area (Sawyer, 1973). The latter approach which utilizes an Aroclor or a mixture

MONS 056276

of Aroclors to duplicate the residue peak pattern is recommended by the AOAC for quantitation of PCB residues in certain foods (Horwitz, 1975). This is a currently accepted and practical way to quantitate PCB residues. Perchlorination of PCBs to decachlorobiphenyl has been suggested as a means to improve precision and consistency in PCB determination (Berg et al., 1971; Armour, 1973). This approach will not improve the accuracy of quantitation, however, because proportions of individual chlorobiphenyls in the PCB residue remain unknown; equal weights of individual chlorobiphenyls of different chlorine content result in different weights of decachlorobiphenyl. Contaminations found in antimony pentachloride, the perchlorination reagent, also detract from this procedure for quantitation purposes (Trotter and Young, 1975). An approach to quantitation which appears to have practical merit as well as offering improved accuracy in quantitation has been advanced by Webb and McCall (1973). The PCB residue is quantitated peak by peak in comparison to reference Aroclors which have been characterized as to the number of chlorines and the fraction of total Aroclor weight represented by each electron capture peak after separation on a widely used (methyl silicone liquid phase) gas chromatographic column. The availability of carefully characterized Aroclors and evaluation in practice are required to fully evaluate the merits of this procedure.

MONS 056277

Precision in the interlaboratory determination of PCBs is slightly less than with the common organochlorine pesticides. In several interlaboratory studies involving biological samples and paperboard containing either added Aroclors or actual residues of PCB the coefficients of variation are about ± 20 percent. The results of studies conducted by the AOAC and the FDA involving samples containing added Aroclors have been mentioned above (Food and Drug Administration, 1971; Burke, 1971; Food and Drug Administration, 1973; Sawyer, 1973); the recoveries of added Aroclors ranged from 74 to 101 percent and coefficients of variation from 9 to 15 percent. The levels of Aroclors added in these studies ranged from about 2 to 8 ppm with the exception of 0.2 ppm Aroclor 1254 added to chicken infant food. With samples containing actual residues, 9 laboratories in the AOAC study reported 9.2 ppm ± 8 percent for a residue in chicken fat and 4.5 ppm ± 20 percent for a residue in Lake Michigan chubs. The residue in the chubs was determined after separation of DDE, DDT, and TDE by column chromatography on silicic acid. In a study by 8 laboratories in cooperation with the International Council for the Exploration of the Sea, PCB residues determined in a fish oil averaged 1.97 ppm ± 47 percent. A much better coefficient of variation, about ± 11 percent, was obtained when the same fish oil was fortified with an additional 10 ppm PCB (International Council for Explorstoin of the Sea, 1974). In an AOAC study of the method for PCB in paperboard 11 laboratories analyzed a paperboard sample manufactured to contain Aroclor 1242 and reported 5.6 ppm ±

MONS 056278

16 percent (Finsterwalder, 1974). The inter- or intra-laboratory precision of the determination of PCB in any sample type is improved by use of the same analytical procedures, especially in quantitation of the residue.

The lower limit of quantitation for PCB residues will vary among laboratories depending upon the objectives of their analyses and upon the particular details of the analytical methods, especially the sensitivity of gas chromatographic detection and the sample size. The quantitation limit achieved will be higher than for organochlorine pesticides because of lower effective detector response to the technical PCB mixtures; 20-30 times more Aroclor 1254 or 30-50 times more Aroclor 1242 than DDE is required for the major peaks to produce the same peak height as DDE in electron capture gas chromatography (Food and Drug Administration, 1968-1975). Other factors which may restrict the attainment of low limits of quantitation and affect analytical reliability are interfering residues in the sample and laboratory contamination. Fish from some locations contain residues of organochlorine pesticides and possibly other contaminants at levels that would cause increasing difficulty as the PCB residue decreases below about one ppm. Contamination of laboratory equipment, reagents, and samples with PCB from containers, previous samples, control runs, and unknown sources, must be carefully guarded against in analyses where a low level of quantitation is necessary, for example with human blood or certain

MONS 056279

environmental samples (Trotter, 1975; Jensen et al., 1972; Giam and Wong, 1972). Perchlorination of the PCB residue to decachlorobiphenyl offers the possibility of about 25 fold increase in sensitivity in the gas chromatographic determination (Armour, 1973). However, contaminants present in antimony pentachloride severely restrict or prohibit application of this procedure to determination of low levels of PCBs (Trotter and Young, 1975). Analytical methods used by the FDA for PCB residues also recover chlorinated napthalenes (Armour and Burke, 1971). If present alone, chlorinated naphthalenes would be recognized by the analyst. However, their presence in admixture with PCBs would probably go unrecognized unless amounts were greater than PCBs or the residue was examined by mass spectrometry. Chlorinated naphthalenes are oxidized by procedures used to differentiate DDE in the presence of PCBs and could be removed from interfering in the PCB determination (Holmes and Walden, 1972).

From examination of procedures used to analyze for chlorinated dibenzofurans in commercial PCB formulations and from analytical studies with chlorinated dibenzodioxins it can be inferred that some chlorinated dibenzofurans may be recovered along with PCBs through analytical methods using Florisil and column chromatography (Vos, et al., 1970; Roach and Pomerantz, 1974; Bowes, et al., 1975; Porter and Burke, 1971). The chlorinated dibenzofurans have gas chromatographic properties similar to the PCBs (Bowes, 1975).

MONS 056280

It is very doubtful, however, that a relatively small amount of chlorinated dibenzofuran in the presence of PCBs would be recognized in the usual analysis for PCBs.

Little study has been made of residue analytical methodology for chlorobiphenylols (hydroxylated chlorinated biphenyls). Procedures used for their determination differ substantially from those used for PCBs (Bache and Lisk, 1973; Zitko et al., 1974). It is not likely that chlorobiphenylols would be recovered through column chromatographic and separation procedures used for PCBs.

In the discussion of PCB residues in foods given in the report of the Human Exposure Group a large share of the information comes from the FDA's surveillance programs. The analytical methodology used in the analyses is described in the FDA Pesticide Analytical Manual, Vol. I, and in Official Methods of Analysis of the AOAC. Quantitation of PCB residues is by gas chromatography with electron capture or halogen specific electrochemical detectors. The total response (area or peak height) for the PCB residue is compared to the total response for the Aroclor reference (or mixture of Aroclors) having the most similar gas chromatographic pattern. Aroclor reference materials are each from a single master lot. Results are reported as ppm of the Aroclor(s) used for the quantitation reference. With certain exceptions the limit of quantitation for PCBs (based on the electron capture detector response to Aroclor 1254) is

MONS 056281

about 0.2 ppm for individual foods and about 0.05 ppm for Total Diet composites. Fish from certain fresh water locations are the food most frequently and consistently found to contain PCB residues. The residues in fish are most often similar in gas chromatographic peak pattern to Aroclor 1254 but usually with higher concentrations of the late eluting (higher chlorination) chlorobiphenyl components.

E. Chlorinated Dibenzofurans

(See Appendix for structural information and definitions)

In considering the potential hazard to humans of the commercial chemical mixtures of chlorinated biphenyls called PCBs, one must consider which if any identified trace contaminant in these complex mixtures might contribute significantly to the overall hazard potential of the mixture. Although there is evidence for the presence of chlorinated napthalenes and possibly chlorinated terphenyls in PCBs, the chlorinated dibenzofuran (Cl-DBF) contaminants are regarded as a greater potential danger for several reasons. In using a chick bioassay to monitor fractionation of commercial PCB mixtrues, it was found (Vos et al., 1970) that the fraction most toxic to chicks, and far more toxic than the other fractions, contained Cl-DBFs and chlorinated naphthalenes. What limited toxicological information there is available on these halogenated naphthalenes, terphenyls and dibenzofurans suggests that only the Cl-DBFs may be more toxic than chlorinated biphenyls by orders of magnitude (Bauer et al., 1961; Moore et al., 1976).

MONS 056282

The extremely high toxicity of some of the chlorinated-dibenzo-p-dioxins (NIEHS Conference 1973; Schwetz, et al., 1973) a class of organic chemicals very similar in structure to the Cl-DBFs, also suggests the latter are likely to be highly toxic. Knowledge of the chemistry and toxicity of various chlorinated dibenzo-p-dioxin structures and the relationship between toxicity and structure, although recently developed, is more complete than for Cl-DBFs. Therefore, findings in the chlorodioxin field provide indicators to assess the difficulties likely to be encountered in the study of Cl-DBFs.

Chlorinated dibenzofuran contaminants have been reported and confirmed in PCB mixtures manufactured in Germany and France (Vos et al., 1970; Bowes, et al., 1975a) in Japan (Roach and Pomerantz, 1974a; Nagayama, et al.[1] 1975) and in the United States (Aroclors) (Roach and Pomerantz, 1974b; Bowes, et al., 1975a). An additional claim that a cl-DBF contaminated an Aroclor was not sufficiently supported by the evidence presented (Curley et al., 1975). In the PCBs of U.S. manufacture, the range of Cl-DBFs found was from dichloro through hexachloro (see references cited above) and sufficient evidence was obtained to show that 2,3.7,8-tetrachloro and 2,3,4,7,8 -pentachlorodibenzofuran are present in Aroclors (Bowes et al., 1975b). That the Cl-DBFs reported were actual contaminants and not artifacts resulting from the experimental procedures used, was considered in two instances (Roach and Pomerantz, 1974b; Nagayama et al., 1975).

MONS 056283

Proper quantitation of the individual Cl-DBF contaminants is very difficult. It requires full structural identification of the Cl-DBF contaminant, availability of the contaminant as a highly purified reference standard for quantitation purposes and evaluation of the procedure used to concentrate and separate the Cl-DBFs, (e.g. from chlorobiphenyls) to determine the capability of the procedure to recover the specific Cl-DBFs in the PCB mixture. Reported attempts to quantitate the Cl-DBFs in PCB suggest contamination levels in the low parts per million range for total chlorinated dibenzofurans (Bowes et al., 1975a; Nagayama et al., 1975). Many of the reported values probably can be taken as minimal in the absence of recovery data.

Although the source of the Cl-DBF contaminants in commercial PCB mixtures has not been determined, several possibilities may be considered. The simplest explanation would be the likely presence of dibenzofuran (parent compound) in the technical grade biphenyl subjected to the chlorination process. From a consideration of the procedures used in the commercial synthesis of PCBs (Hubbard, 1964) various chlorinated biphenyls, if substituted in the ortho and ortho-prime positions with hydroxy groups and/or chlorine atoms, might ring close to form the furanoid ring by dehydration or dehydrochlorination.

It is important to recall that Cl-DBFs have been reported as contaminants also in other chemicals of commercial importance. Samples of pentachlorophenol (Schwetz et al., 1974) as well as

MONS 056284

lower chlorinated phenols (Firestone et al., 1972) and hexachlorobenzene, (Villanueva et al., 1974) have been found to contain a range of Cl-DBFs. The ppm level of Cl-DBFs in certain pentachlorophenols examined by Schwetz and co-workers (1974) was considerably higher than the highest level thus far reported in commercial PCB mixtures; the chlorine level in the reported dibenzofuran contaminants of pentachlorophenol ranged from hexa to octa.

It is now known that "PCB residues" obtained from environmental samples usually do not simply represent an easily identified original commercial PCB mixture. For example, there is evidence from the study of electron capture gas chromatograms that certain chlorinated biphenyl compounds are preferentially lost or concentrated in passing through environmental media. This has led to recent studies of the toxicity, metabolism and physiological effects of specific, individual chlorinated biphenyl compounds, synthesized by routes intended to yield a single compound of known structure. In addition to permitting the development of structure-activity correlations, these studies presumably were intended to avoid complications in interpreting results from the testing of the commercial chlorinated biphenyl mixtures (Vos and Notenboom-Ram, 1972), now known to contain toxic Cl-DBFs as contaminants. Interpretaion of results from the testing of individual chlorinated biphenyl compounds, however, may not be entirely straight forward. In the Ullmann coupling reaction of

MONS 056285

2,4,5-trichloroiodobenzene. It has been reported that the highly toxic 2,3,7,8-tetrachlorodibenzofuran is formed in 3% yield in addition to the expected 2,2'4,4',5,5'-hexachlorobiphenyl product (Moron, et al., 1973). This hexachlorobiphenyl is a significant constituent of PCB mixtures (Sissons and Elti, 1971; Tas and Kleipool, 1972; Jensen and Sundström, 1974) and has been singled out for biological studies by several workers (Vos and Notenboom-Ram, 1972; Johnstone et al., 1974; Hansell and Ecobichon, 1974). Careful purification and, if necessary, chemical analysis of symmetrical chlorobiphenyls prepared by the Ullmann coupling reaction (for dibenzofuran content) is suggested as a prerequisite to biological testing of the biphenyl.

There are two other aspects of the chemical relationship betwen chlorinated biphenyls and Cl-DBFs that need to be mentioned. Both deal with possible environmental alteration. Evidence has been obtained for photochemical conversion of certain chlorinated biphenyls to Cl-DBFs in very low yields (Crosby and Moilanen, 1973). Although the scope of this study was limited, formation of Cl-DBF was shown to occur under conditions of sunlight irradiation and in the laboratory using sunlight-simulating conditions. Two different ortho-chlorinated biphenyls (the 2,5-dichloro and 2,2',5,5'-tetrachloro) were claimed to produce approximately 0.2% steady-state yields of a monochloro DBF. The limited reports regarding the photochemical decomposition of Cl-DBFs (Crosby and Moilanen, 1973; Hutzinger et al., 1973) indicate relatively

MONS 056286

rapid destruction of the compounds may take place in the environment. Since reductive dechlorination is a major route of photochemical alteration of Cl-DBFs, the possibility of dechlorination of highly chlorinated DBFs to more toxic DBFs of lower chlorine content must be considered. The extent to which specific chlorinated biphenyls, found as major constituents of commercial PCB mixtures, can be photochemically converted to Cl-DBFs can only be determined by further experimental studies.

Analysis of the Yusho oil (the rice oil contaminated by PCB-containing heat exchange fluid implicated in the Japanese "Yusho" poisoning incident) by Nagayama et al., (1975) for Cl-DBF content, led to a value of 5 ppm Cl-DBFs. This value is about 300 times the Cl-DBF level expected in the Yusho oil if one simply assumes contamination of the oil by Cl-DBFs to be proportional to the Cl-DBF level found in unused Kanechlor 400 (the Japanese PCB mixture claimed to have been used as the heat exchange fluid) and to the level of PCB in the Yusho oil. This led Kuratsune et al., (1975) to suggest that Cl-DBF levels increased in the heat exchange fluid through use. Going a step further, these questions may be raised: Is Cl-DBF concentration increased, generally, in PCB-containing heat exchange fluids through use? Does such increase occur in PCB-containing transformer fluids or electrical capacitors through use?

A critical review is needed of the published data upon which Kuratsune's suggestion is based (Nagayama et al., 1975), particularly

MONS 056287

since the results from a recent analysis of a portion of Yusho oil (Trotter, 1976) raises questions about the concentration of chlorinated biphenyls in the Yusho oil. Further laboratory studies suggested by such a review and analyses of selected, used PCB-containing industrial fluids might help to answer the above questions.

Considering the Cl-DBFs as the toxicologically most significant class of chemical impurities in the PCB mixtures, the questions may be asked: what portion of the observed effects from animal and human exposure to PCB mixtures can be attributed to the Cl-DBFs? It is doubtful this question can be answered in any quantitatively explicit way. There are analytical problems in identifying and measuring accurately the amounts of chlorinated dibenzofurans in various PCB mixtures. Even though only a relatively few of the 135 possible chlorinated dibenzofurans may be identified in PCB mixtures, the toxicity of these compounds can be expected to vary with both number and ring position of chlorine atoms in the Cl-DBF molecule. Toxicity data on specific, high purity Cl-DBFs is very limited because the compounds themselves are not readily available. In addition to these, essentially chemical, difficulties, toxicological judgements would be difficult to make. To begin to evaluate the toxicological "burden" to be placed on Cl-DBF contaminants in commercial PCB mixtures, a practical approach would be to use analytical procedures that can recover and quantitate specific Cl-DBF compounds known to be highly toxic. For example, 2,3,7,8-tetrachlorodibenzofuran, already reported by Bowes (1975b) as

MONS 056288

present in PCBs, can serve as a measure of potential hazard until further toxicity data for Cl-DBFs become available.

To date, there have been no published, positive findings of Cl-DBFs in environmental samples or in foods. Analytical procedures to detect Cl-DBFs in such samples should be developed, tested and applied to appropriate samples. If chlorinated dibenzo-p-dioxin residue analytical findings can be taken as an indicator, procedures suitable for parts per trillion detection of Cl-DBFs will be required and quantitation will be difficult.

No clear evidence for the presence of chlorinated dibenzo-p-dioxins in commercial PCB mixtures has been reported. A recent publication suggesting the presence of such contaminants in some PCBs and in a synthesized chlorinated biphenyl (Ax and Hansen, 1975) almost certainly misinterpreted the significance of the experimental finding related to the dioxins.

III. Chemistry of Brominated Biphenyls

A. Comparison of Brominated Biphenyls with Chlorinated Biphenyls

Unlike PCBs, the chemistry and stability of PBBs have not been well studied and documented in the literature. It is difficult to assess the stability and the extent of possible chemical conversion of PBBs in the environment. PBBs can be compared chemically to the PCBs. Since both bromine and chlorine are

MONS 056289

halogens, PBB and PCB chemistry should be similar in some respects. Bromine, however, is a better leaving group in chemical reactions than chlorine. The described laboratory experiments concerning PBB alkaline hydrolysis and photolysis show that bromine is more labile than chlorine under comparable reaction parameters. PBBs may, therefore, be less stable in the environment if PBBs were under the same physical reaction parameters in the environment as PCBs. The physical state and other physical reaction parameters of PBB and PCB residues in the environment can determine reactivity. Liquids and vapors often react more readily than solids. Aroclors are liquids, though some Aroclors are extremely viscous at room temperature. Some PCBs, especially the lower chlorinated components, are volatile. FireMaster BP-6, however, is a solid and has an extremely low vapor pressure. The production, distribution, and usage of PBBs has not been as wide spread as PCBs. PBBs, unlike PCBs, may not be physically located in a position for chemical reaction. FireMaster BP-6 has been extensively used, for example, in thermoplastics, such as typewriter and business machine housings (Karst, 1974). FireMaster BP-6 has little tendency to migrate from the thermoplastic into which it is incorporated. The major portion of the products into which FireMaster BP-6 is incorporated is assumed to be buried eventually in refuse dumps. PCBs, on the other hand, are often used in transformers and capacitors and can be exposed to high temperatures which can accelerate possible chemical reactions. PCB residues are found in many diverse locations

MONS 056290

throughout most of the world. Many of these locations may offer conducive settings for chemical and metabolic conversions. PCB residues in a body of water may react photolytically, whereas PBB residues buried in a refuse dump would not be in a position to absorb light.

B. Non-metabolic Alteration of Brominated Biphenyls

Oxidation and Hydrolysis

The stability of PBBs to oxidation has not been studied and documented in the literature. FireMaster BP-6 is unstable to alkaline hydrolysis. After refluxing FireMaster BP-6 with 2% KOH in ethanol, GLC chromatograms show erratic degradation of hexabromobiphenyl (DET,DO,DCT,DCH,1976), the major component of FireMaster BP-6. The possible rate of PBB hydrolysis in the environment under milder conditions is not known.

Photochemistry

Recent analysis of FireMaster BP-6 by GCMS using an OV 101 column demonstrated 12 peaks whose mass spectrum corresponds to 2 pentas, 4 hexas, 4 heptas and 2 octas. However, about 50 percent of the material is 1 hexa isomer with the next most abundant component being the hepta isomer at about 20-25 percent. Nuclear magnetic resonance and chemical studies have identified the principal component of Firemaster BP-6 as 2,2'4,4'5,5'-hexa-bromobiphenyl (Anderson et al., 1974; Sundström et al., 1976).

MONS 056291

The photolytic rates of reactivity of FireMaster BP-6 and 2,2'4,4'5,5'-hexachlorobiphenyl in methanol solutions irradiated with 3000 Å light were compared (Ruzo and Zabik, 1975). The rate of reaction of FireMaster BP-6 was determined presumably by the decrease in the GLC peak of hexabromobipenyl. Hexabromobiphenyl was found to be seven times as reactive as the corresponding PCB. Hexabromobiphenyl was found to undergo photolytic reductive debromination in methanol yielding penta- and tetrabromobiphenyls and also > 1% methoxylated product. Dimethoxy tetrabromobiphenyl was identified as a product by mass spectrometry. The highly efficient photoreactivity of PBB may be due to: 1. enhanced intersystem crossing to a triplet state due to vibronic coupling with the bromines, 2. steric interference due to the bromines, and 3. the relatively low carbon-bromine bond energy. The aromatic carbon-bromine bond energy is 71 kcal/mole vs the aromatic carbon-chlorine bond energy of 86 kcal/mole (Kerst, 1974). As with PCBs (Ruzo, et al., 1974), presumably ortho halogens preferentially cleave in PBBs upon photoexcitation.

The possible rates and extent of photolytic reactivity of PBBs in the environment are not known. The photochemistry of PBBs has not been studied in the vapor or solid states. Since FireMaster BP-6 has an extremely low vapor pressure, vapor state photochemistry in the environment would be extremely limited. The extent of thin film PBB photochemistry in the environment may also be limited. PBB contamination and resulting

MONS 056292

photochemistry in river waters, except in the vicinity of PBB production facilities, may be limited. PBBs incorporated into thermoplastics, which are presumably buried eventually in a refuse dump, are not likely to absorb much light for photolytic reaction.

C. Brominated Biphenyls: Determination of Residues

Polybrominated biphenyl (PBB) residues are analyzed for in foods by methods quite similar to those used for organochlorine pesticides and polychlorinated biphenyls (PCBs) (Food and Drug Administration, 1968-1975; Fehringer, 1975; Food and Drug Administration, 1976). Recoveries of PBB added to samples *in vivo* through these procedures is over 80 percent. A method to improve extraction efficiency of PBB residue from dry, high fat animal feeds has been reported (Fehringer, 1975).

The major difference from methods for organochlorine pesticides is in the gas chromatographic determination which is done at higher temperatures or on columns with low liquid phase loads because of the lower volatility of PBBs. Methyl silicones or Silar-10C, the liquid phases most frequently used, separate the technical PBB, FireMaster BP-6, into 3-9 peaks depending on the chromatographic conditions selected. PCBs and organochlorine pesticides which would be recovered through extraction and cleanup procedures together with PBBs have earlier retention times under these conditions and are separated from the

MONS 056293

major PBB constituent, hexabromobiphenyl. The retention times of hexabromobiphenyl and decachlorobiphenyl are not greatly different on methyl silicone liquid phases but are widely separated on Silar-10C. Elution of the Florisil column used for cleanup of extracts with petroleum ether rather than a mixture of petroleum ether and ethyl ether separates PBBs from most organochlorine pesticides prior to gas chromatography and improves cleanup for the PBB determination.

PBB residues are detected and quantitated with the electron capture gas chromatography detector. Quantitation is based on comparison of the size of the hexabromobiphenyl peak in the residue to the size of the hexabromobiphenyl peak in a known weight of the reference material, FireMaster BP-6. FireMaster BP-6 is a technical mixture containing 60-70 percent hexabromobiphenyls (Sundström and Hutzinger, 1976). The accuracy of residue quantitation is affected by any change in the composition of the residue from that of the reference technical PBB mixture. The identity of PBB residues can be confirmed by thin layer chromatography, photochemical alteration, halogen-specific gas chromatographic detection, and by different retention times on Silar-10C and methyl silicon gas chromatographic columns (Food and Drug Administration, 1976; Fehringer, 1974; Fehringer, 1975; Erney, 1975). Mass spectrometry has also been used to confirm the identity of the PBB residue (Ayers, 1975).

MONS 056294

The limit of quantitation for PBB residues, including capability for residue identity confirmation, is about 0.05 ppm in fats and 0.01 ppm in non-fats (Food and Drug Administration, 1976). Formalized interlaboratory studies have not been reported for analytical methods for determination of PBB residues.

D. Possibility of Brominated Dibenzofurans in Commercial PBB Mixtures

There has been no report, so far, of the finding of brominated dibenzofuran (Br-DBF) compounds in commercial PBB mixtures. Examination of FireMaster BP-6 for possible contamination by Br-DBF is in progress in several laboratories.

MONS 056295

METABOLISM AND BIOCHEMICAL TOXICITY OF PCBs and PBBs

I. Effects of PCBs on Biochemical Functions

One of the major biochemical effects of PCBs is the induction of microsomal enzymes in the liver. Risebrough et al., (1968) suggested that PCBs had the capability to induce the activities of microsomal enzymes. Subsequently, Street et al., (1969) demonstrated the induction of liver enzymes in rats by PCB's. Since then, a great number of articles have been published on this subject.

The enzyme systems studied have included mainly hydroxylases, N- and O-demethylases and nitroreductases, and to a lesser extent nonspecific carboxylesterase, bromosulphophthalein-glutathione conjugating enzyme, p-nitrophenol UDP-glucuronyl transferase and EPN-detoxification systems.

The induction of microsomal enzymes by commercial PCBs has been demonstrated perorally in rabbits (Villeneuve et al., (1971), rats (Litterst et al., 1972a) and primates Allen et al., 1974) and via intraperitoneal injection (Bickers et al., 1972) and skin application in rats (Bickers et al., 1975). Values reported for the threshold of enzyme induction by PCB's vary between 0.5-25 ppm. (Litterst et al., 1972b; Villeneuve et al., 1971; Turner et al., 1974).

The time-course of microsomal eyzyme induction was studied by Litterst et al., (1974) in rats. They found that significant levels of enzyme induction occurred after 7 days of feeding PCBs

MONS 056296

in the diet. A single oral dose in rats resulted in maximum enzyme activities at 24 hours. Bickets et al., (1974); 1975) have shown that maximum induction occurred within 2-6 days in rats after cutaneous exposure to Aroclor 1254 or microscope immersion oils containing 30-45% chlorine.

In order to study the effect of chlorine content of PCB's on enzyme induction, different commercial PCBs were investigated. Litterst et al., (1972b) studied PCBs with chlorine contents from 42 to 60%. In rats the maximum activity of demethylase was observed with the 54% chlorine PCB's; maximum activity for nitroreductase was obtained with the 60% chlorine PCBs. Chen et al., (1973) reported that 54% chlorinated PCBs gave maximum response of demethylase in rats. Similar results were reported by intraperitoneal injection of various PCBs in rats (Ecobichon et al., 1974). Goldstein et al., (1975) and Iverson et al., (1975) studied the effects of Aroclor 1016 and Aroclor 1242 on enzyme induction in rats. Both PCBs have approximately 42% chlorine, but the content of the penta-, hexa-, and hepta-chlorine isomers of Aroclor 1016 is reduced to 10% of that in Aroclor 1242. Their results showed that Aroclor 1242 was a much more potent inducer than Aroclor 1016 in female rats. In comparing Aroclor 1232, Aroclor 1248 and Aroclor 1260, Schmoldt et al., (1974) found that Aroclor 1260 was the most potent enzyme inducer in rats.

The synthetic and isomerically pure PCBs have been investigated for enzyme induction, Chan et al., 91973b) found that both

MONS 056297

2,3,4,5- and 3,5,3',5'-tetrachlorobiphenyls induced enzyme systems in male rats, but no significant differences were found between the two isomers. Johnstone et al., (1974); Ecobichon et al., (1975); Ecobichon (1975) compared the effects of position of chlorine atoms on the ring. They found that enhanced induction of mono-oxygenases was observed for PCBs having chlorine atoms substituted at the 4- and 4'- positions irrespective of chlorination at other positions. Substitution at the 2-position was next in importance followed by substitution at the 3-position. They concluded that the position of chlorination was as important as the degree of chlorination.

There are two types of enzyme inducers being reported in the literature. One group to which phenobarbital belongs resulted in increased cytochrome P-450 content, as well as increased benzpyrene hydroxylase and ethylmorphine demethylase activities in the liver. The second group includes polycyclic hydrocarbons. This group stimulates the formation of cytochrome P-448 and an increase in hydroxylation but not demethylation. Alvares et al., (1973) reported that rats treated with PCBs produced an increase in cytochrome P-448, hydroxylase, as well as demethylase. Therefore, it appears that PCBs display induction behavior typical of both groups.

Most of the enzyme induction studies were evaluated in vitro. Several workers indeed have observed enzyme induction effects in vivo by demonstrating shortened barbiturate sleeping times (Bickers et al., 1972; Johnstone et al., 1974; Villeneuve et al., 1972);

MONS 056298

Commercial PCBs are known to contain small amounts of dibenzofurans (DBF). DBFs were found to be 170 times as potent as PCBs in inducing enzymes.

Alvales & Kappas (1975) reported the induction of hydroxylase and demethylase in placenta, as well as in the fetus, when pregnant rats were treated with PCBs Vainio (1974) reported that Clophen A50 enhanced hydroxylase 5-fold in the lung and 8-fold in the kidney microsomes, whereas the microsomes from duodenal mucosa exhibited no enhancement. Benzpyrene hydroxylase activity in skin was also increased by PCBs (Bickers et al., 1975).

Vos and Koeman (1970) found that several tissues of PCB-treated chickens were strongly fluorescent under ultra violet light. They suggest that PCBs could induce chemical porphyria in chickens. Later, they demonstrated that mitochondrial ALA Synthetase activity increased 20-fold and fecal porphyrin levels were significantly increased in Japanese quail treated with PCBs (Vos, 1971; Vos et al., (1972). They suggested that the porphyria caused by PCBs is due to the increase of ALA synthetase, followed by overproduction of porphyrins. Similar results in rats were obtained by Goldstein et al., (1974), and Bruckner et al., (1974). Goldstein et al., (1974) suggested that the induction of ALA synthetase is probably not the primary cause of PCB-induced porphyria because of the delayed onset of porphyria as compared with ALA synthetase induction. They suggest that the ALA synthetase in the liver may be related to the increase of cytochrome P-450 content. This

MONS 056299

idea was supported by Grote et al., (1975). Aroclor 1242 was found to be a more potent inducer of liver porphyria than Aroclor 1016 (Goldstein et al., 1975). Both Aroclors have similar chlorine content, but Aroclor 1242 contains 9% homologs with five or more chlorines while Aroclor 1016 contains only 1% of such homologs. Sinclair and Granick (1974) showed that cultured liver cells respond rapidly to PCBs in accumulation of uroporphyrin. Simon et al., (1974) suggested that porphyria may be due to the destruction of the phospholipid structure of cell membrane, resulting in increased permeability of the substrate. Hirayama et al., (1974) observed hypo-bilirubinaemia in Yusho patients and suggested that the induction of bilirubin UDP-glucuronyltransferase may be responsible for the low plasma bilirubin content. However, recently Bastomsky et al., (1975) showed that the lowering of serum bilirubin concentration by PCBs is due to the reduced binding of bilirubin to plasma protein rther than to the enzyme induction. Wit (1972) has shown that PBBs which are similar to PCBs are potent heptoporphy-rinogenic chemicals.

The chemical and structural similarity of DDT to PCBs led Jefferies et al., (1972) to study the effects of PCBs on thyroid functions. They found an increase in thyroid weight in PCB-treated black-backed gulls as compared to controls. Hurst et al., (1974) found no clear-cut effect of PCBs on thyroid gland size in quails. Byrne et al., (1975) reported that PCBs increased

MONS 056300

thyroxine levels in the blood and also promoted peripheral degradation of thyroxine in female minks. Bastomsky (1974) found a 4 to 5-fold increase of biliary excretion of thyroxine in rats treated with PCBs. PCBs also elevated iodine uptake by the thyroid and reduced serum PBI concentration. Recently Bastomsky and Wise, (1975) showed similar results in rats due to either intraperitoneal injection or skin application of microscope immersion oil. Walker (1975) found that inclusion of Aroclor 1254 in fish food resulted in an increase in fish thyroid activity.

The effects of PCBs on ATPase and oxidative phosphorylation have been examined by a number of investigators. $Na^{+}K^{+}$-ATPase and Mg^{++} ATPase from several fish tissues were found to be inhibited by PCBs. (Yap et el., 1971; Cutkomp et al., 1972; Desaiah et al., 1972; Koch et al., (1972). Further study showed that in vitro data were not in agreement with the in vivo data (Yap et al., (1974). The inhibition of ATPase in fishes by PCBs was also reported by Kinter et al., (1972) and by Davis et al., (1972).

Sivalingan et al., (1973) investigated the effects of PCB on the oxidative phosphorylation of rat liver mitochondria. They showed that the mode of inhibition appears to be different with PCBs of different chlorine content. PCBs with low chlorine content inhibited energy and electron transfer but PCBs with high chlorine content not only inhibited energy and electron transfer but also had an uncoupling effect. LaRocca et al.,

MONS 056301

(1975) showed that the administration of commercial PCBs, as well as purified isomers to rats inhibited the total ATPase activity in liver, kidney and brain tissues.

Pardini (1971) has demonstrated that a marked inhibition of respiratory enzyme systems occurred when heavy beef heart mitochondria were exposed in vitro to numerous PCBs. Chesney and Allen (1974) showed that the addition of PCBs to rat liver mitochondria in vitro caused an inhibition of oxidative phosphorylation and respiration. Feeding rats with PCBs at the 1000 ppm level increased the oxidative phosphorylation in liver mitochondria, however no effect was seen at the 100 ppm level.

Sharp et al., (1974) reported that the inhibition of beef brain and rabbit kidney Na^+K^+-ATPases can be reversed or prevented by phosphatidylserine or phosphatidylinositol, but not by phosphatidychioline and phosphatidylethanolamine. They suggested that acidic phospholipids are required to stabilize the enzyme and thereby overcome the effect of PCBs.

Risebrough et al., (1968) suggested that PCBs had the capacity to enhance steroid-hydroxylating enzyme activity, thus affecting estradiol metabolism. In addition, certain low chlorine containing PCBs have estrogenic activity which is reflected by an increase in glycogen content of the uterus of immature rats (Bitman and Cecil, 1970). Similar results were obtained by Fumiko (1972) in rats (Bitman and Cecil, 1970). Similar results were obtained by

MONS 056302

Fumiko (1972) in rats and by Orberg, and Kihlstrom (197) in mice. Orberg et al., (1972) found that the estrous cycle of the mouse increased in length after a single injection of PCBs. Lincer and Peakall, (1973) reported an increased rate of metabolism of estradiol in vitro in the liver of American kestrels after the feeding of PCBs. Similar results were also reported in white leghorn cockerels and pullets (Nowicki and Norman 1972). Platonow and Funnell (1971) demonstrated that feeding PCBs to cockerels resulted in a decrease in testicular and comb growth. Decreased urinary excretions of estrogen and dehydroepiandrosterone were also observed in cockerels when PCBs were administered in high doses (Platonow et al., 1972). Ecobichon and Mackenzie, (1974) studied the uterotropic activity of commercial and isomerically pure PCBs in rats. They found significant changes with a number of PCB mixtures but experiments with the isomerically pure PCBs were inconclusive. Changes were observed with 2-chloro and 2,2-chlorobiphenyls. Nelson (1974) reported that PCBs are effective inhibitors of the binding of ^{3}H-estradiol to the rat uterus cytosol fraction in vitro. Recent data showed that PCB feeding caused neither significant chromosomal damage nor arrest in the rate of spermatogenesis in male rats (Dikshith et al., (1975).

Flick et al., (1965) noted enlarged adrenals and small spleens in PCB-treated white leghorn cockerels. Wassermann and Wassermann (1972) and Wessermann et al., (1973) reported that rats receiving 200 ppm PCBs showed an increase in plasma corticosterone levels.

MONS 056303

These changes were concimitant with morphological changes in the zona fasciculata of the adrenal gland which indicated increased activity. Sanders, et al., (1974) have shown similar increases in serum corticosterone in mice fed 60 to 1000 ppm PCBs.

It is known that DDT decreases the utilization of dietary carotene and liver storage of vitamin A in rats and cattle (Phillips, 1963; Phillips, and Hidiroglou, 1965). Villeneuve et al., 1971) reported that Aroclor 1254 but not Aroclor 1221 reduced the liver concentration of vitamin A in pregnant rabbits. Decreased liver vitamin A concentrations were found in male and female rats and also Japanese quail after PCB feeding (Cecil et al., 1973). However, the laying female quail did not appear to be affected.

The effects of PCBs on vitamin E have been studied by Combs et al., (1975). Their results indicated that dietary PCBs increased the incidence of exudative diathesis in chicks and that this increase can be overcome by increasing dietary vitamin E or selenium. The vitamin D mediated calcium metabolism was found to be altered by the oral administration of PCBs to chickens (Wong et al., 1974).

II. The Comparative Biochemical Toxicity of Aroclor 1254 and Firemaster BP-6

PCB's have been reported to affect a large number of biochemical systems. To the extent that it has been reported, PBBs

MONS 056304

affect most of these same systems in a similar fashion. However, quantitative and possibly qualitative differences do not appear to exist in the response of some biological parameters. These include thyroid hyperplasia (Norris et al., 1973) and microsomal enzyme induction (Farber and Baker, 1970).

A preliminary study was conducted to compare the relative effect of Aroclor 1254 (Aro) with Firemaster BP-6 (FM) (Garthoff et al., 1975). This was set up as a broad survey to give an estimate of the relative biological activity of these chemicals in may different systems and pin point areas where further more definitive work should be done.

Table 1 summarizes the time sequence of the various parameters studied. Adult male rats were fed either 0, 5, 50, or 500 ppm Aroclor 1254 or Firemaster BP-6, mixed in the diet, for 2, 3, or 5 weeks.

Due to the large number of parameters examined and the time restraints placed on the study, the experimental design did not permit a totally unambiguous comparison between all of the effects caused by Aro and FM. Some apparent differences in biochemical responses determined at sacrifice may have been partly due to the fact that, for the three-week study, rats were exposed to FM for 48 hours longer than rats were exposed to Aro. Although it is felt that under the experimental conditions, this effect should be small, critical comparison of Aro and FM toxicity should be

MONS 056305

considered tentative until smaller experiments are conducted which can eliminate this difference in exposure time. The following evaluations were made based on the assumption that this two day difference had no effect.

Some parameters showed no consistent or dose related change with either chemical at any level. These included relative kidney weight and testes weight, hematology, SGPT, SGOT, plasma BUN, plasma corticosterone, the rate of liver protein synthesis per g tissue, liver dry weight, lyophilizable kidney, liver, or testes, the incidence of chromosome abnormalities and the number of cells in mitosis from bone marrow of spermatogenial cells.

Growth and adipose tissue weight were equally depressed by Aro and FM when fed over a three week period at 500 ppm. The growth effect appeared to be due primarily to decreased food efficiency.

Three parameters were more sensitive to Aro than to Fm. These included the decreased liver protein and liver RNA per g tissue and the decreased plasma glucose.

On the other hand, six parameters were more sensitive to FM. These included liver growth, increased liver lipid (total lipid, cholesterol, phospholipid, and neutral lipid), elevated plasma cholesterol, increased microsomal enzyme activities, impaired mitochondrial function, and decreased liver RNA synthesis.

MONS 056306

Disruptions of the redox and energy states of the cell were produced by dietary exposure to Aro or FM. These effects were indicated by studies wit isolated mitochondria and frozen clamped liver. In the latter case the concentrations of adenine nucleotides, pyridine nucleotides, and glycolytic intermediates were determined. On a molar basis, hexabromobiphenyl was reported to be about five times more potent than Arochlor 1254 in causing some increased microsomal enzyme activities (Farber and Baker, 1974). A three-fold difference was observed between Firemaster BP-6 and Arochlor 1254 (Table 3).

A pathology team autopsied rats after both three and five weeks exposure to Aro and FM (Kasza et al., 1975). Pathological analysis of the liver of rats fed Aro or FM for three weeks showed no abnormalities on gross observation. No histopathological lesions were seen in controls, Aro groups, or the 5 and 50 ppm FM groups, after three weeks. In the 500 ppm FM group, minimal vacuolation and focal hepatitis were each seen in one rat. After five weeks exposure, gross observation of the liver indicated an increasing incidence of enlargement, friability, and prominent lobular pattern with increasing dose of both chemicals. Microscopically the 5 ppb Aro group appeared normal while 2/7 rats fed 5 ppm FM had minimal cytoplasmic degeneration. All rats had this lesion at 50 and 500 ppm Aro or FM, with increasing cellular lipid. This lesion was recent, centrilobular and coalescing with no change in fibrous tissue or glycogen. The histopathology was qualitatively similar but slightly more severe in rats fed FM

MONS 056307

than in those fed Aro and is consistent with biochemical findings. The biochemical findings were all made after three weeks exposure at which time histopathological changes were not evident. Even at five weeks the histopathology was not severe enough to alter most of the clinical chemistry in the rat.

In most parameters evaluated, FM either caused an effect at a lower dose level or caused an effect in a shorter time or caused a greater effect at the same dose level, expressed as ppm in the diet, as Aro. When expressed on a molar basis FM becomes even more effective than PCB. Exceptions to this observation were noted at the 500 ppm dose level where differential effects of Aro and Fm were often eliminated.

A summary of the major effects seen are found in Tables 2, 3, and 4. In all but one case the maximum response of a parameter occurred at the highest dose level. Microsomal enzyme activities were, in general, greater at the 50 ppm level and the decline at 500 ppm relative to the 50 ppm level was more marked with FM.

III. Metabolism of PCB and PBB Mixture

Generally, the tissues from animals and man containing PCB from environmental exposure have GLC patterns resembling those of PCB mixtures with more than 50% chlorination. This is in marked contrast to the major manufactured products that generally contain 42% or less chlorine. While one cannot rule out the possiblity that differential uses favored introduction of more highly

MONS 056308

TABLE 1

PARAMETERS EVALUATED IN STUDY

EXPOSURE TIME

(WEEKS)

PARAMETERS	TWO	THREE	FIVE
Body weight	x	x	x
Liver weight	x	x	x
Other tissue weights		x	
Mitochondrial respiration	x		
Liver composition		x	
Liver protein synthesis		x	
Liver RNA synthesis		x	
Liver Microsomal enzyme activities		x	
Liver intermediary metabolites		x	
Serum cholesterol		x	
Serum enzymes		x	x
Plasma corticosterone		x	
Plasma protein		x	
BUN		x	x
Gross pathology		x	x
Histopathology		x	x
Cytogenetic analysis			x

MONS 056309

TABLE 2

RELATIVE BIOCHEMICAL RESPONSE TO Aro AND FM AFTER TWO WEEKS DIETARY EXPOSURE

	LEL[1] ppm		Maximum Effect % Control	
	Aro	FM	Aro	FM
PARAMETERS				
Liver weight / Body weight	500	50	170	183
Mitochondrial respiration	500	500	152	192

[1]LEL = lowest effective level that showed a dose-response relationship.

MONS 056310

TABLE 3

RELATIVE BIOCHEMICAL RESPONSE TO Aro AND FM
AFTER THREE WEEKS DIETARY EXPOSURE

	LEL[1] ppm		Maximum Effect % Control	
	Aro	FM	Aro	FM
PARAMETERS				
Body weight gain	50	500	52	52
Liver weight / Body weight	50	50	203	214
Liver dry wt.	NE[3]	50	NE[3]	107
Liver lipid	50	5	138	156
Liver cholesterol	500	50	226	232
Liver protein	500	NE	84	NE
Liver RNA	5	50	79	84
Liver DNA	5	5	58	60
Liver RNA synthesis	500	5	78	86

MONS 056311

TABLE 3 (Cont'd)

PARAMETERS	LEL[1] ppm Aro	LEL[1] ppm FM	Maximum Effect % Control Aro	Maximum Effect % Control FM
para-Nitrobenzoate reductase activity	50	5	288[2]	520[2]
Plasma glucose	50	NE[3]	79	NE[3]
Plasma cholesterol	500	5	178	256

[1]Lowest effective level that shows a dose response relationship.

[2]Maximum effect occurred at 50 ppm dose.

[3]NE = No effect

MONS 056212

TABLE 4

RESPONSE OF RELATIVE LIVER WEIGHT TO Aro AND FM AFTER FIVE WEEKS DIETARY EXPOSURE*

PARAMETER	LEL ppm		Maximum Effect % Control	
	Aro	FM	Aro	FM
Liver weight / body weight	500	50	171	216

* Legend - see Table 3.

MONS 056313

chlorinated materials in the environment, the observation has led to the general belief that the less chlorinated components are more readily metabolized.

The environmental observations have been confirmed in a large number of experimental feeding studies in a number of mammalian and avian species. The absence or diminished concentration of the early eluting peaks have been reported in rats (Grant et al., 1971a, Curley et al., 1971), rabbit (Grant et al., 1971b), cow (Fries et al., 1973a), quail (Bailey and Bunyan, 1972), and hens (Fries et al., 1973b).

The peaks not present in tissues and products generally are the early eluting peaks that correspond to the PCBs with lower degrees of chlorination. The observations are consistent with the belief that the rate of metabolic attack on PCBs decreases with increasing chlorination. Studies of the single PCB homologs of various degrees of chlorination have shown that those with five or less chlorine atoms are more radily metabolized and excreted than the PCBs with higher chlorination (Berlin et al., 1975; Hutzinger et al., 1972; Melvas and Brandt, 1973). The position of chlorine substitution also affects the retention and elimination of single homologs (de Freitas and Norstrom, 1974). Metabolism of individual PCBs will be discussed more thoroughly in another section.

MONS 056314

Among the various food species there appears to be one exception to the general rule that the less chlorinated PCBs are hydroxylated. The PCB residues in fish closely resemble the PCB to which the fish was exposed. There was little change in the relative concentrations of the various homologs (Stalling and Mayer, 1972). Consistant with this observation it has been found that mono-, di-, and tetrachlorobiphenyls are not metabolized by trout (Hutzinger et al, 1972).

Among the food producing animals (cattle, chickens) concentrations of PCB residues in milk and eggs reach a "steady state" after approximately 8 to 12 weeks of continuous intake (Fries et al., 1973a; Fries et al., 1973b). The ratio of residue levels in food products to levels in the animal's diet are greatest with PCBs of 54% chlorination. The residue levels in milk are approximately 4 times the residue level in the cow's diet. In hens, residue levels in eggs are approximately equal to the diet while residue level in the body tissue (fat basis) is approximately 6 times the dietary level. The ratio of residue level in product and tissues to intake is lower with PCBs of greater than 54% chlorination. Since it is unlikely that there is greater metabolism of the most highly chlorinated PCBs, the results suggest that the absorption for the GI tract is lower for the more highly chlorinated PCBs (Smith et al., 1976). PCBs of four or fewer chlorines rarely occur in animal products when the animals are fed environmentally realistic levels.

MONS 056315

Much less is known about the metabolism of PBB than PCBs. However, the observations that have been reported tend to be consistent with the reports on PCB metabolism.

While the PBB of most immediate concern for human health (BP-6, Michigan Chemical Co.) is a multicomponent mixture like the PCBs, it is much simpler than the ordinary commercial PCB mixtures. The major component is a single hexabromobiphenyl accounting for over 60% of the total material. The only other component occurring in significant amounts is a single heptabromobiphenyl.

In general, the measures of retention in edible tissues and excretion in edible products are similar for the hexabromobiphenyl and the hexachlorobiphenyls (Fries et al., 1976; Fries and Marrow, 1975). Heptabromobiphenyl retention and excretion is only 1/10 of that of the hexabromobiphenyl. In cattle that have been heavily contaminated with BP-6, the heptabromobiphenyl was barely detectable (milk or tissues) 6 to 8 months after the time of ingestion even though the hexabromobiphenyl level was over 1000 ppm (Fries et al., 1975). Since the heptabromobiphenyl is not more likely to be metabolized by the animal than the hexabromobiphenyl, it is reasonable to assume that its absorption is lower or its elimination through bile is greater than the hexabromobiphenyl component. Over 60% of octabromobiphenyl is excreted in the feces as the parent compound when it is fed to rats (Norris et al., 1974). This observation supports the conclusion that there is less absorption with increasing halogenation.

MONS 056316

IV. The Metabolism of Individual PCBs

Quantitative studies of the distribution of PCBs indicate that most, if not all, PCBs which contain six or fewer chlorine atoms are efficiently absorbed from the gut of higher animals (Albro and Fishbein, 1972; Matthews and Anderson, 1975a; Van Miller et al., 1975). Furthermore, only about one-tenth of the absorbed dose is excreted prior to metabolism to more polar compounds (Matthews and Anderson, 1975a; Van Miller et al., 1975; Balin et al., 1975). A number of the less chlorinated PCBs (i.e., mono-, di-, tri-, and tetrachlorobiphenyls) are readily metabolized and excreted by birds and mammals. There is little evidence that fish can metabolize any PCB.

It has been adequately demonstrated that a number of mammalian species and the pigeon can readily degrade and excrete monochlorobiphenyls, primarily 4-chlorobiphenyl (Matthews and Anderson, 1975a; Block and Cornish, 1959; Hutzinger et al., 1972; Safe et al., 1975a,b,c). However, the rate of metabolism drops sharply as the number of chlorines per molecule increases. For example, the rate of metabolism and excretion of several PCB isomers has been shown to decrease as the number of chlorines increases in the following order: 4-chlorobiphenyl 4,4'-dichlorobiphenyl 4,4'-dichlorobiphenyl 2,5,2',5'-tetrachlorobiphenyl 2,4,5, 2',5'-pentachlorobiphenyl (Matthews and Anderson, 1975a; Van Miller et al., 1975; Matthews and Anderson, 1975b). The rate

MONS 056317

of metabolism and excretion of 4,4'-dichlorobiphenyl is approximately one-half that of 4-chlorobiphenyl. In each case the PCBs are metabolized primarily by hydroxylation and conjugation with glucluronic acid (Matthews and Anderson, 1975a; Balin et al., 1975). The importance of the route of excretion, i.e., urine or feces, is determined primarily by the degree of chlorination. Approximately 60, 30, and 7 percent of a single dose of a mono-, a di-, and a pentachlorobiphenyl, respectively, were excreted in the urine of rats. The remainder of the dose was excreted in the feces (Matthews and Anderson, 1975a).

Once the number of chlorine atoms on the biphenyl molecule reaches four, the position of the chlorine atoms becomes a more important factor in determining the rate of metabolism and excretion (Matthews and Anderson, 1975a; De Freitas and Norstrom, 1974). As was observed with the chlorinated benzenes (Jondorf et al., 1955; Jondorf et al., 1958) those PCBs which have two adjacent unsubstituted carbon atoms are metabolized and excreted more rapidly than PCBs having the same degree of chlorination, but which do not have two adjacent unsubstituted carbon atoms (Matthews and Anderson, 1975a). It is also likely that, as with the chlorinated benzenes, the absence of two adjacent unsubstituted carbon atoms has a greater effect as the degree of chlorination increases. For example, the initial half-life of 3,5,5',5'-tetrachlorobiphenyl is only about two-fold greater

MONS 056318

in the rat (Matthews and Tuey, 1976) than that of 2,5,2',5'-tetrachlorobiphenyl in the same species (Van Miller et al., 1975). On the other hand, the initial half-life of 2,4,5,2',4',5'-hexachlorobiphenyl appears to be infinite, whereas the initial half-life of 2,4,5,2',5'-pentachlorobiphenyl is only about two days (Matthews and Anderson, 1975a). This particular hexachlorobiphenyl has been shown to be in the highest concentration of any PCB found in the adipose tissue of the Swedish population (Jensen et al., 1974).

The importance of two adjacent unsubstituted carbon atoms to the metabolism of PCBs may be derived from their effect on the formation of arene oxide intermediates during the metabolism of these compounds by the hepatic mixed-function oxidases. Evidence for the formation of arene oxide intermediates in PCB metabolism was first provided by Gardner et al., (Gardner et al., 1973) in their study of the metabolism of 2,5,2',5'-tetrachlorobiphenyl and in a later study of 4-chlorobiphenyl metabolism by Safe et al., (1975c) which was specifically designed to provide evidence of an arene oxide intermediate. Metabolism of PCBs which do not have two adjacent unsubstituted carbon atoms by hepatic mixed-function oxidases, via direct hydroxylation of the biphenyl molecule and/or the formation of an arene oxide between a chlorinated and an unsubstituted carbon atom could occur, but each of these mechanisms would be expected to be slower than arene oxide formation between two unsubstituted carbon atoms (Daly et al., 1972; Jerina and Daly, 1974). The latter mechanism would be expected

MONS 056319

to result in dechlorination and/or shifting of a chlorine atom (Daly et al., 1972). The metabolism of selected hexachlorobiphenyls has been reported (Jensen and Sundstrom, 1974; Hutzinger et al., 1974; Hass, unpublished), and in two incidents (Hutzinger et al., 1974; McKinney et al., unpublished) dechlorination and/or chlorine shifting was observed, but in all cases the rate of metabolism was quite slow.

Thus, those PCBs which are more readily metabolized and excreted may also more readily form arene oxides. Certain arene oxides have been implicated as potential carcinogens (Daly et al., 1972; Jerine and Daly, 1974) as have certain PCB formulations (Ito et al., 1973; Kimbrough and Linder, 1973; Kimbrough et al., 1975), but there is no published evidence which indicates that the less chlorinated PCB formulations have been so rigorously tested.

MONS 056320

ANIMAL TOXICOLOGY

Introduction

A number of reviews on different aspects of the toxicology of the PCBs have been written recently and no effort has been made in this report to give a complete outline of the animal toxicology (Kimbrough, 1974; Peakall and Risebrough, 1975; Nelson et al., 1972; Fishbein, 1974).

The material summarized in this report consists predominantly of more recently published information on toxic effects of PCBs in mammals and experimental studies in birds. Since Nelson et al. (1972) discussed the toxic effects produced in fish by PCBs extensively, the reader is referred to this and the other reviews cited above for information on toxicity of PCBs in this species.

The emphasis of the present review was placed on chronic toxic effects wherever possible. Unfortunately, very little information was available for halogenated dibenzofurans and the brominated biphenyl mixtures.

Acute Toxicity

Polychlorinated Biphenyls (PCB)

The acute oral LD_{50} of the PCBs in rats, rabbits and mice ranges from 1 to 10 g/kg body weight (Tables 5,6). There is some indication that young animals may be more sensitive than adults,

MONS 056321

and females more sensitive than males (Table 5). According to the classification used by the American Industrial Hygiene Association (Hodge & Sterner, 1949), the acute toxicity of the PCBs can be classed as slightly toxic (0.5 to 5 g/kg body weight) to practically non-toxic (5-15 g/kg body weight).

The symptoms associatiated with administration of a toxic dose of PCB (Aroclor 1242) in rats consist of diarrhea, diminished exploratory behavior, decreased response to pain stimuli, chromodacryarrhea, adipsia, oliguria, anorexia, erythema of limbs, followed by ataxia, coma and death. Death occurred up to 14 days following administration of the toxic dose. 24 hours after administration of a single toxic dose of Aroclor 1242, gross pathology indicated all organs appeared normal except the liver and kidneys. No inflammation was observed in the abdominal and intestinal mucosa. Histopathological findings showed large discrete sudanophilic vacuoles in the hepatocytes. Widely scattered foci of tubular epithelial cells were present in the kidneys (Bruckner, et al., 1973). However, Kimbrough (1974) reported ulceration of the gastric and duodenal mucosa in rats after single oral doses of 3,000 mg/kg Aroclor 1254 or 1260.

Only one metabolite of PCB is known to have been tested for the acute LD_{50}. The 5-OH metabolite of 2,4,3'4'-tetrachlorobiphenyl was known to be more toxic than the parent compound (0.43 g/kgBW/2.15 g/kgBW (Table 5). Dermal toxicity of PCB is summarized in Table 7.

MONS 056322

Polybrominated Biphenyls (PBBs)

Polybrominated biphenyls show a low order of acute toxicity to the rat (Table 8). In the case of chicks, a 37% mortality was reported when PBB was administered in the diet at a level of 400 ppm, for 15 days. Seven out of eight guinea pigs fed 50 ppm dietary PBB died (Vos and VanGerderen, 1973). Effects noted included cortical atrophy of the thymus, adrenal enlargement, marked depletion of the follicles and periarteriolar lymphocytes sheath of the spleen.

Chlorinated Dibenzofurans

See subacute toxicity.

Chlorinated Napthalenes

See end of chapter.

MONS 056323

TABLE 5

Compound Tested	Species	Route	g/kg body wt. LD_{50}	Reference
Aroclor 1254	Rat(Adult-Sherman Strain)	oral	4-10	Linder, et al. (1974)
" 1260	Rat(Adult-Sherman Strain)	oral	4-10	"
" 1254	Rat(weanling " ")	oral	1.295	"
" 1260	Rat(weanling " ")	oral	1.315	"
" 1254	Rat(female " ")	Intravenous	0.358	"
" 1221	Rat(female " ")	oral	4.00	Panel on Hazardous Trace Substances (1972)
" 1262	Rat(female " ")	oral	11.3	
" 1260	Rat	oral	4.25	Bruckner, et al. (1973)
" 1254	Rat(Wistar-30-day-old M-F)	oral	1.3	Grant & Phillips (1974)
" 1254	Rat(Wistar-60-day-old M-F)	oral	1.4	"
" 1254	Rat(Wistar-120-day-old M)	oral	2.0	"
" 1254	Rat(Wistar-120-day-old F)	oral	2.5	"
Kaneclor-400	Rat(Wistar Strain - Male)	oral	1.30 (ml/Kg)	Ikeda, et al. (1970)
Kaneclor-400	Rat(Wistar Strain - Female)	oral	1.14 (ml/Kg)	
Kaneclor-400	Mice(CFI Strain - Male)	oral	1.875 (ml/Kg)	
Kaneclor-400	Mice(CFI Strain - Female)	oral	1.57 (ml/Kg	
Kaneclor-300	Rat(Wistar Strain - Male)	oral	1.15	
Kaneclor-300	Rat(Wistar Strain - Female)	oral	1.05	
BP-200 (biphenyls of dicloride and below	Mice(dd-strain-female)	oral	6.36	Kitamura (1972)
2,4'-dichlorobiphenyl	Mice(dd-strain-female)	oral	7.86	"
trichlorobiphenyl	Mice(dd-strain-female)	oral	3.06 - 4.25	
biphenyl of trichloride and below	Mice(dd-strain-female)	oral	9.27	"
2,4,3',4'-tetrachlorobiphenyl	Mice(CFI strain)	Intraperitoneal	2.15	Yamamoto & Yoshimura (1973)
5-OH derivative of 2,4,3'4'-tetrachlorobiphenyl	Mice(CFI strain)	"	0.43	"
2,3,4,3'4'-pentachlorobiphenyl	Mice(CFI strain)	"	0.65	"

MONS 056324

TABLE 6

Oral LD_{50} (Rat) (Panel on Hazardous Substances, 1972)

Compound Tested		g/Kg body wt.
Aroclor 1221	(undiluted)	3.98
Aroclor 1232	(undiluted)	4.47
Aroclor 1242	(undiluted)	8.65
Aroclor 1248	(undiluted)	11.0
Aroclor 1260	(50% soln in corn oil)	10.0
Aroclor 1262	(50% soln in corn oil)	11.3
Aroclor 1268	(50% soln in corn oil)	10.9

MONS 056325

TABLE 7

Skin MLD_{50} Rabbits (Panel on Hazardous Substances, 1972)

Compound tested		g/Kg body wt.	
Aroclor 1221	(undiluted)	>2.000	<3.169
Aroclor 1232	(undiluted)	>1.26	<2.0
Aroclor 1242	(undiluted)	> .794	<1.269
Aroclor 1248	(undiluted)	> .794	<1.269
Aroclor 1260	(50% soln in corn oil)	>1.26	<2.0
Aroclor 1262	(50% soln in corn oil)	>1.26	<3.16
Aroclor 1268	(33.3% soln in corn oil)	>2.5	

MONS 056326

TABLE 8

LD_{50} Polybrominated Biphenyls

Compound	Species	Route	LD_{50}	Reference
Firemaster BP6	Rat	Oral	21.5 g/Kg	Hill Top Research, Inc. (1970) fo Michigan Chemical Company
Octabromobiphenyl	Rat	Oral	More than 2 g/Kg	Aftosmis, et al., 1972
Octabromobiphenyl	Bobwhite Quail	Oral	More than 12.5 g/Kg	"
Hexabromobiphenyl	Rabbit	Dermal	More than 5 g/Kg	"
Octabromobiphenyl	Rabbit	Dermal	More than 10 g/Kg	"

MONS 056327

SUB-ACUTE AND REPRODUCTIVE EFFECTS OF PCB'S, PBB'S AND CHLORINATED DIBENZOFURANS

A. Mammalian Sub-acute Effects of PCBs

In contrast to the acute toxicity (oral and dermal) of the PCBs which is of relatively low order when the substances are administered as a single dose, aspects of sub-acute toxicity of both the PCBs and individual chlorinated biphenyls appear to be of far greater concern exhibiting species sensitivity and cumulative toxic effects following continuous exposure at low levels.

When groups of mice were given toxic rice bran oil containing 1,600 ppm PCBs or 5000 ppm of PCB mixture with 48% chlorine, the toxicity response was similar in the two groups (Nishizumi 1970).

In a group of rats fed 2000 ppm of Phenochlor DP-6, deaths occurred between the 12th and 26th days with enlarged livers, atrophy of the spleens and a progressively induced hepatic porphyria was noted (Vos and Koeman, 1970). Administration of 1000 ppm of Arochlor 1254 in the diet of rats resulted in deaths between the 28th day and 53rd day (100% mortality) of feeding (Tucker and Crabtree 1970), 50% mortality at the same dosage in an 8-month study was reported by Kimbrough et al. (1972).

MONS 056328

Two groups of 52 male Sherman strain weanling rats were fed 100 ppm Aroclor 1242 (6.6-3.89 mg Aroclor/kg body weight) and 100 ppm Aroclor 1016 (6.9-3.5 mg Aroclor/kg body weight) respectively for 6 months and 54 rats were fed ground chow. Groups of 4 rats were sacrificed at intervals. Microscopic examination of the liver showed evidence of lipid accumulation, enlarged liver cells, inclusions in a number of livers and hemorrhage and necrosis in one animal per experimental group each. No difference in effect between the two compounds was noted (Burse et al., 1974).

Rats given 100 mg Aroclor 1242/kg body weight by stomach tube every other day for 3 weeks showed histopathological changes only in the liver and kidneys as foci of sudanophilic vacuolation but no overt sign of toxicity (Bruckner et al., 1973).

Rats (Sprague-Dawley) given 100 ppm of 2,5,2', 5'-tetrachlorobiphenyl in the diet for 3 weeks showed less liver hypertrophy and fewer biochemical changes than rats given a similar dose of Aroclor 1248 (Allen et al., 1975).

Administration of 100 mg/kg body weight of Aroclors 1242, or 1254 orally to rabbits once a week for 14 weeks resulted in enlarged livers with apparent destruction of the rough endoplasmic reticulum and atrophy of the uteri, while Aroclor 1221 did not cause this effect (Koller and Zinkl, 1973).

MONS 056329

The unusual sensitivity of mink to PCBs was reported by Aulerich et al., (1973) who noted 100% mortality within 6 months following administration of 30 ppm PCB (10 ppm each of Aroclor 1242, 1248 and 1254) to adult mink. Feeding of Aroclor 1254 at levels of 5 and 10 ppm in the diet for 4 months led to a dose-dependent retardation of weight gain of growing female mink. Platonow and Karstad (1973) produced 100% mortality in mink when they fed this species 3.6 ppm Aroclor 1254 in the diet for 105 days.

Rhesus monkeys are also very sensitive to PCBs (McNulty, 1975). For example, adult female rhesus monkeys (Macaca Mulatta) have been fed diets containing 2.5, 5.0 and 25.0 ppm of Aroclor 1248 over periods ranging from 2 months for the higher PCB levels to 1 year for the two lower doses (Allen et al., 1974; Barsotti and Allen, 1975). Animals on the 25 ppm dose developed facial edema, alopecia and acne within 1 month, and 1 of 6 animals had expired as a result of PCB intoxication 2 months after removal from the diet. The total intake of PCBs ranged from 250 to 400 mg/animal. As was the case with the higher doses, these animals developed anemia, hypoproteinemia, bone marrow atrophy and severe hypertrophic hyperplastic gastritis. PCB concentrations in subcutaneous adipose tissue averaged 127 ppm at termination of experimental diet and 34 ppm 8 months later. Surviving animals continued to show clinical signs and lesions of PCB intoxication 2 years following PCB exposure (Allen, 1975).

MONS 056330

When adult rhesus monkeys were fed diets containing 2.5 and 5.0 ppm Aroclor 1248 for 1 year, the females of the group developed periorbital edema, alopecia, erythema and acne from lesions that involved the face and neck within 1 to 2 months (Allen et al., 1974; Allen, 1975). Although males consumed more PCB, they exhibited only moderate periorbital edema and erythema. An abnormal dysplastic growth pattern of the gastric mucosa was also noted in male rhesus monkeys fed 300 ppm Aroclor 1248 for 3 months (Allen and Norback, 1973).

Cygomegalous or squirrel monkeys fed different dietary levels of Kanechlor 400 showed weight loss, palpebral edema, enlargement of the liver and pneumonia. The lowest effective total dose was 300 mg/kg bw given over 20-32 weeks (Nishizumi, 1970).

A rhesus monkey fed 3 ppm Aroclor 1242 for 8 months died spontaneously and on autopsy was found to possess a gastric lesion manifested by extensive down growth of the epithelium deep into the subserosa and into the muscular wall of the stomach itself (McNulty, 1975).

One monkey fed 2,4,4'-trichlorobiphenyl and another fed 2,4,5,4'5'-pentachlorobiphenyl at 10 ppm for 90 days were reported clinically well and autopsies showed no positive findings (Oregon State University, 1975; McNulty, 1975).

MONS 056331

Third-litter sows received feed containing Aroclor 1242 at a concentration of 20 ppm throughout gestation and nursing. Treated sows differed significantly from control sows in the number of live pigs farrowed. Treated sows also had more mummified fetuses. Performance of live-born pigs was not affected by feeding PCB to the sows during nursing. Pathologic changes in the sows included hypertrophy of the liver and erosion of the stomach. Slight atrophy of the spleen and thyroid gland were observed in pigs from treated sows (Hansen et al., 1975).

Dermal toxicity studies in rabbits of technical PCB samples that contain an average of 60% chlorine (Phenoclor DP6, Clophen A60, and Aroclor 1260) as well as fractions containing tetra and pentachlorodibenzofuran have been recently described by Vos and Beems (1971). PCB-induced skin lesions were hyperplasia and hyperkeratosis of the epidermal and follicular epithelium following application of 118 mg of the three PCBs (5 times per week for 38 days) in the back skin of adult female New Zealand rabbits. Histopathology of the liver included centrolobular degeneration, centrolobular liver cell atrophy, focal necrosis, and cytoplasmic hyalin degeneration. PCB-induced kidney lesions were hydropic degeneration of the convoluted tubules and tubular dilation with the presence of casts. Definitive hyperplasia and hyperkeratosis of the follicular epithelium of the ear skin were seen after the topical application of fractions of Phenoclor and Clophen, while the fraction from Aroclor caused a minimal hyperplasia and hyper-

MONS 056332

keratosis of the follicular epithelium. Other effects elicited by the dermal application of the PCBs including thymus atrophy and lymphopenia as well as elevated excretion of fecal coproporphyria and protoporphyria.

From the response of the back skin and the liver of the rabbit to the three PCB mixtures, and from the response of the ear to the 25% diethyl ether-hexane fractions it was concluded that there were definite quantitative differences in toxicity, at least between the samples used in the above study and prior studies. The extent to which these samples are representative of the normal commercial output has not been established and emphasizes the difficulty in the evaluation of toxicity data of PCBs in which the samples may differ in the amount and nature of toxic impurities.

Vos & Notenboom-Ram (1972) compared the toxicity of Aroclor 1260 with a single isomer 2,4,5,2',4',5'-hexachlorobiphenyl in New Zealand rabbits. Dermal applications of a total 120 mg Aroclor 1260 (5 times/week for 28 days) resulted in early macroscopic skin lesions. The lesions in a 2,4,5,2',4',5'-hexachlorobiphenyl group of rabbits treated similarly appeared later and were less severe.

When groups of 5 male mice were fed dietary levels of 10, 30, 100 and 300 ppm of the PCB isomers 3,4,5,3',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl and 2,4,6,2',4',6'-hexachlorobiphenyl

MONS 056333

for 4 weeks it was noted that the 3,4,5,3',4',5', isomer of hexachlorobiphenyl caused death in mice at a dietary intake of 10 ppm (2.1 mg/kg bw) within 36-47 days of exposure while consumption of the other two isomers resulted only in death at a dietary level of 300 ppm (64.3 mg/kg bw). Body weight was reduced at the lowest dose for the 3,4,5,3',4',5', isomer and only at the highest dose for the other two isomers. Atrophy of the thymus and hepatomegaly was noted at the lowest dose in the mice receiving the 3,4,5,3',4',5', isomer and only this isomer produced experimental porphyria.

B, Avian subacute toxic effect of PCBs.

Chickens fed 10 ppm Aroclor 1242, or 100 ppm Aroclor 1254 showed diminished growth but a dietary level of 100 ppm Aroclor 1260 did not produce this effect (Kaplinger, et al., 1971).

Symptoms of PCB poisoning in birds consist of tremor, ataxia, ruffling, loss of feathers. Edema of the subcutaneous tissues, and fluid accumulation of the abdominal and thoraxic cavities are characteristic signs at autopsy. These findings are responsible for this disease being designated chick edema disease. Several outbreaks of chick edema disease caused by PCBs have been reported (Firestone 1973, Kohanowa 1969a, 1969b).

Chicks (one day old white leghorn cockerels) were fed the following PCB isomers: 2,3,6,2',3',6'- hexachlorobiphenyl 2,4,6,2',4',6',hexachlorobiphenyl, 2,3,4,2'3',4'-hexachlorobiphenyl,

MONS 056334

2,3,6,2',3',6', hexachlorobiphenyl and 2,4,5,2',4',5' hexachlorobiphenyl for 21 days at a level of 400 ppm. The isomer 2,4,5,2',4',5' hexachlorobiphenyl was also fed at the dietary level of 100 ppm. The 3,4,5,3',4',5' isomer was the most toxic causing death of all chicks within 11 days after onset of exposure. These chicks had chick edema disease, atrophy of the thymus and loss of subcutaneous and visceral adipose tissue. The other isomers also caused weight loss the 2,4,6,2',4',6' isomer being the least effective. Liver enlargement was noted for all groups the 2,3,6,2',3',6' being the least effective and the 2,4,6,2',4',6' the most effective. The microscopic findings made in the liver were most pronounced in the group fed the 2,4,6,2', 4',6' isomer. (Since the chicks fed the 3,4,5,3',4',5' isomer died relatively early in the experiment pathological findings in the livers of these chicks are not comparable to those made for the other isomers.)

When chicks were fed 5% rice oil which had produced Yusho they developed chick edema in 17 days. Similar symptoms were produced when chicks were fed 400 ppm Kaneclor 400 (Goto et al., 1969).

C. Mammalian Reproductive Effects of PCBs

Oral administration of 0.025 mg/day of Clophen A60 in peanut oil to NMRI-mice for 10 weeks lengthened the estrus cycle from 6.6 $\pm$ 2.5 days to 8.7 $\pm$ 4.3 days (Orberg and Kihlstrom, 1973; Kihlstrom et al., 1973). Females treated analogously for 62 days

MONS 056335

and mated with untreated males exhibited a significant decrease in the implantation rate from 87.0% in the controls to 79.5%. Male castrated mice were given 0.25 gm PCB (Clophen A 60) in peanut oil/day by stomach tube for 28 days and 70 μg testosterone propionate daily on days 19-28. The dry weight of the seminal vesicles after this treatment period was compared to castrated male mice given testosterone but not PCB and was found to be significanlty reduced.

Groups of 10 NMR I female mice were injected subcutaneously with 50 mg/kg body weight of Clophen A 60 in peanut oil or only the vehicle on the day of parturition and thereafter once weekly for 3 weeks. The 209 offspring were bred when reaching sexual maturity by mating them in pairs as follows: 17 pairs of controls, 19 pairs where the males had received Clophen A 60 as a suckling, 23 pairs where the female had received Clophen A 60 as sucklings and 23 pairs were both sexes had received Clophen A 60 as sucklings. The controls had an average of 11.5 offspring per litter while the parents where both mates received PCBs as sucklings had 8.8 offspring per litter. The resorption rate for both groups was the same but the number of implantations ware reduced. Reproduction was not affected where only one mate received the PCB (Kihlstrom et al.,1975).

Keplinger et al.,(1972) reported low mating indices and decreased survival of pups for rats receiving Aroclor 1242 at 100 ppm, and decreased survival of pups receiving Aroclor 1254

MONS 056336

at 100 ppm. No reproductive effects were found with Aroclor 1260 at 1, 10, or 100 ppm, or with Aroclor 1242 or 1254 at 1 or 10 ppm. These studies suggest that in mammals reproductive effects decrease with increasing chlorination.

Sherman rats were exposed to polychlorinated biphenyls, Aroclor 1254 and Aroclor 1260. Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups per litter than the controls in the F1b and F2 generations (Linder et al., 1974). The 100 ppm exposure level of Aroclor 1254 increased mortality in the F1b offspring and markedly decreased mating performance of the F1b adults. The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 21-day-old F1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F1 and F2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. Histological changes in the liver and increased liver weights were observed in adult rats exposed to the higher levels. Pregnant rats given Aroclor 1254 at the rate of 100 mg/kg/day on days 7-15 of gestation produced grossly normal litters, but only 30.1% of the pups survived to weaning. Reproduction and pup survival were not affected at dosage rates of 50 mg Aroclor 1254/kg bw/day or 100 mg Aroclor 1260/kg bw/day.

MONS 056337

Rabbits have been found to be more sensitive to PCBs than rats in regard to fetotoxic and reproductive effects (Villeneuve et al., 1971). In rabbits, no effects were reported at dosages of 6.25 or 10 mg Aroclor 1254 /kg/day, but at dosages of 12.5 to 50 mg/kg/day, fetotoxic effects were noted. Abortions, maternal death and stillborns occurred, but no consistent skeletal abnormalities were found.

Ringer et al., (1972) found that 1 ppm of Aroclor 1254 caused a slight reduction in reproductive success in mink, but at 5 ppm Aroclor 1254 complete reproductive failure was noted. In the same study 12 mink were fed a diet containing 30% Coho Salmon from Lake Michigan. None of the mink whelped while 11 of 12 controls whelped.

Female adult rhesus monkeys fed Aroclor 1248 at a level of 25 ppm for 2 months noted at beginning of the 5th month experienced either fetal resorption or abortion during their second month of pregnancy (evidenced by regression in uterine size and reestablishment of menstruation (Allen et al., 1974). A third animal carried her fetus to term. This infant's weight was considerably lower than the average rhesus infant (375g vs 544 $\pm$ 101g) and had 25 μg PCB/g/(ppm) of adrenal and adipose tissue. Adult female rhesus monkeys fed 2.5 and 5.0 ppm Aroclor 1248 were bred to control males following the establishment of a relatively steady tissue level of PCB at 6 months. Following 3 mating, 12.5% of the 5 ppm group and 37.5% of the 2.5 ppm group was pregnant

MONS 056338

compared to 90% in the control group (Allen, 1975). The conception rate of females bred to PCB-fed males was equally as great as that in females bred to control males.

D. Mammalian Sub-Acute and Reproductive Effects of PBBs

Male Sprague Dawley rats maintained on diets containing 0, 0.1, 0.01 or 1% octobromobiphenyl for 30 days did not exhibit overt toxicity during this period. Gross pathological studies revealed enlarged livers and some kidney changes (petechial hemorrhage, enlargement), while histopathological changes in the 1% group included liver lesions consisting of centrilobular cytoplasmic enlargement and vaculation and kidney lesions consisting of hyaline degenerative cytoplasmic changes (Aftosmis et al., 1972). Thyroid hyperplasia was observed in all rats in all test groups.

Rats were fed 100 and 1000 ppm octabromobiphenyl in their diets and then a number were sacrificed after 2 and 4 weeks of feeding and after 2,6 and 18 weeks of recovery on an octabromobiphenyl-free diet. The hepatocytes were markedly enlarged in rats fed 100 ppm and 1000 ppm of the PBB after 2 weeks of treatment. Histopathologic changes were characterized by hepatocellular hypertrophy with the pathologic changes localized mostly in the centralobular zone (Aftomis et al., 1972b). The cytoplasmic inclusions were more pronounced in the livers of rats fed 1000 ppm than 100 ppm of the PBB. There was some evidence of recovery since the livers of the 100 ppm level animals returned almost to normal 2 weeks after

MONS 056339

withdrawal of the compound.

Preliminary 28-day feeding studies in rats with octabromobiphenyl at 100 and 1000 ppm demonstrated liver enlargement, hepatocellular alterations with cytoplasmic inclusions and accumulative effects of bromine in the fat, liver and muscle (Aftosmis et al., 1972).

Norris et al., (1974) demonstrated hematological changes, liver enlargement and liver and kidney lesions at all levels of octabromobiphenyl (10, 000, 1000 and 100 ppm) in the diet of rats. When three groups of primagravid rats fed diets containing 100, 1000 or 10,000 ppm of octabromobiphenyl (BB-8) from day 6 through 15 of pregnancy and the pups delivered on day 20, anasarca was observed in one fetus at each of the two highest levels and gastroschisis was observed in another fetus at each of these levels. No other gross effects were observed in either the mothers or the pups and dose-related levels of bromine were found in the fetuses (Aftomis et al., 1972a).

When 1 g/kg of octobromobiphenyl was applied in corn oil under occuluded conditions to rabbits for 5 hrs/day for 10 days over a 2-week period, liver enlargement was found while at 0.1g/kg no enlargment was found (Aftosmis, et al., 1972b).

Hexabromobiphenyl (Firemaster BP-6) fed to pregnant Sprague-Dawley rats and Swiss/ICR mice (100 and 1000 ppm) resulted in a dose dependent decrease in mean fetal weight. (Number of pregnant

MONS 056340

mice: 16 controls, 9 received 100 ppm and 12 received 1000 ppm. Number of pregnant rats: 12 controls, 7 received 100 ppm and 6 received 1000 ppm). Exencephaly was produced in the offspring of the mice that received both 100 (3/121)and 1,000 ppm (2/174 dosages while cleft palate (4/87) and defective kidneys (2/87) were noted in offspring at the 1,000 ppm level. Nonpregnant mice fed 1,000 ppm PBBs for 11 days showed marked increase in liver size and weight (Corbett et al., 1975).

Cows from the Halbert herd received feed containing PBB at a level of 2914 ppm at a consumption rate of approximately 15 lbs/head/day (200 cows) or 8 lbs/head/day (200 cows) for a period of approximately 16 days. Early signs of toxicity included anorexia, decreased milk production, increased frequency of urination, lacrimation, some lameness, and shrinking of the udder of recently freshened cows. Upon removal of the contaminated feed appetite improved. Cows bred 4-6 weeks prior to onset came back in heat suggesting early resorptions.

Later signs exhibited by the cows included hematomas, abscesses, weight loss particularly in high producing cows, abnormal hoof growth, rough hair coat, alopecia, thickening of skin, dystocia, lack of udder development at freshening, metritis, and negligible milk production in cows freshening 3-4 months post-exposure. Cows also exhibited a lack of wound healing, general weakness and were highly susceptible to stress. Six months post-exposure non-lactating cows showed depression of

MONS 056341

appetite, prepartum weakness, failed to develop signs of labor and died without calving. Gross pathology included liver and renal degeneration, and hematomas and abscesses in the peritoneal and thoracic cavities.

Twelve 6 to 18 month-old heifers and bulls were offered the contaminated feed ad libitum. Five of the animals were dead within 6 weeks. Younger animals became prostrate, went through a short period of coma and died. Signs and lesions included testicular atrophy, abdominal adhesions, and massive liver abscesses. At 5 months only 2 animals remained alive. These became anorexic, would consume only milk and developed signs of hyperkeratosis over the entire body (Jackson and Halbert, 1974; Welborn, 1975).

The health status of 16 herds of dairy cattle exposed to low levels of polybrominated biphenyl (PBB) was compared with that of 15 control herds. Milk production of the contaminated herds was not significangly changed in 1972, 1973, and 1974, and was not significantly different from that of control herds in the same years. Mortality of adult cows and calves, the percentages of cows culled from the herds because of old age and low production, disease, or sterility, and the general health conditions were similar in the two groups. Serum concentrations of calcium, glucose, and cholesterol in contaminated herds were significantly different from those of the control herds, but the relationship to PBB exposure needs further investigation (Mercer et al., 1976, In Press).

MONS 056342

A study consisting of an investigation of records and herd history of herds accidentally exposed to PBBs was also done. Significant findings included a decrease in production of 20 to 50%; severe weight loss at calving time; sterility; decreased growth rate in young animals; evidence of soreness and stiffness; poor response to therapy of common diseases; prolonged wound healing; abnormal hoof growth; calf losses; and malformed calves. (Deming, 1975).

Polybrominated biphenyl was administered to four cows at a dose rate of 10 mg/head/day for 60 days. Although the study was not designed as a toxicology study, retrospective evaluation of the data provided no evidence of clinical problems among test animals during the period of exposure to PBB nor for a year thereafter (Fries and Marrow, 1975).

E. Chlorinated Dibenzofurans - Mammalian Toxicity

It is presently assumed because of our experience with the chlorinated dibenzodioxins, that the 2,3,7,8-tetrachlorodibenzofuran is the most toxic compound of this group of chemicals, while the dibenzofuran itself or the octadibenzofuran have little inherent toxicity on an acute basis (Kimbrough, 1974).

Bauer et al., in 1961 demonstrated the toxicity of mixtures of tri and tetrachlorodibenzofurans. A single dose of 1 mg or 0.5 mg/kg body weight given orally to rabbits caused severe and often fatal liver necrosis. Application of this material to the rabbit

MONS 056343

ear caused severe hyperkeratosis at the application site.

The single oral LD_{50} for tetrachlorodibenzofuran for guinea pigs is between 5 and 10 μg/kg. Symptoms of toxicity were severe weight loss, atrophy of the thymus and spleen. Hemorrhages were observed in the adrenals, urinary bladder and single cell necrosis was noted in the liver. C. D. Strain rats given as much as 1000 μg/kg bw of the TCDF failed to show any symptoms of toxicity and microscopic examination of their tissues did not reveal any abnormalities. Similarly oral single doses of up to 6000 μg/kg body weight of TCDF failed to elicit any toxic effect in C57Bl mice. A subcutaneous dose of 6000 μg/kg bw given to these mice produced weight loss, hepatomegaly and atrophy of the thumus but no fatalities (Moore et al., 1976).

While these findings indicate that in mice TCDF is 30 fold less toxic than TCDD, the overall estimated difference in toxicity between TCDD and TCDF is about tenfold (Bauer et al., 1961; Kimbrough, 1974; J. G. Vos, personal communication).

F. Chlorinated Dibenzofurans - Avian Toxicity

When 1 day old chicks were started on daily oral doses of 5 μg/kg TCDF daily most of them died within 11.5 days. One of 6 chicks died given daily oral doses of 1 μg/kg bw. Symptoms of toxicity at the low as well as the high doses consisted primarily of weight loss, decreased food consumption and general unthriftyness. At autopsy the most striking findings were subcutaneous edema, ascites and hydropericardium and atrophy of the thymus.

MONS 056344

An inflammatory exudate was noticed on microscopic examination in the lung and the pericardial surface of the heart showed inflammatory changes. Congestion and hemorrhage into the gastrointestinal tract was also observed (Moore et al., 1976).

G. Brominated Dibenzofurans - Mammalian Toxicity

When daily doses of 4 μg/rabbit of 2,3,7,8-tetrabromodibenzofurans were applied to the rabbit ear for 5 days giving a total dose of 20 μg/rabbit or about 5-6 μg/kg bw, the rabbits (4) developed hyperkeratosis of the treated ear and areas of liver cell necrosis (Kimbrough, personal communication). No other information is presently available on the toxicity of brominated dibenzofurans.

H. Longterm Toxicity Including Tumorogenesis

Chlorinated biphenyls

Mice

When male BALB c/J mice were fed Aroclor 1254 for 11 months at a dietary level of 0 or 300 ppm (49.8 mg/kg body weight) 22 of 50 mice in the experimental group and 24 of 50 in the control group survived. The livers of the test animals were markedly increased in size, and showed a qualitative increase in porphyrin. A total of 9 experimental mice showed 10 neoplastic nodules (hematomas, hyperplastic nodules) in their liver ranging in size from 0.1 - 0.5 cm in diameter. Other morphological changes observed in the livers of the experimental group including pleomorphism,

MONS 056345

areas of necrosis, and foci of adenofibrosis. (Kimbrough and Linder, 1974). Neither tumors nor the other morphological changes were noted in the controls. Only one of 24 surviving male BALB c/J mice fed Aroclor 1254 for 6 months followed by a control diet for 5 months had a hepatoma (neoplastic nodule) measuring 0.3 cm in diameter.

Ito et al., (1973) fed male dd mice 500, 250, 100 and 0 ppm Kanechlor 500, 400 and 300 in the diet respectively. After one year, hepatocellular carcinomas were observed in 5/12 mice fed Kanechlor 500 at a dietary level of 500 ppm, the other 7 mice in this group had nodular hyperplasia (neoplastic nodules?). No metastases were observed. Feeding α, β, or γ hexachlorocyclohexane (BHC) isomers with or without Kanechlor 500 at a dietary level of 250 ppm to mice for 24 weeks did not produce any tumors in the liver of animals receiving only the PCB (Ito et al., 1973). Liver tumors were observed in mice receiving 250 ppm α BHC alone or 250 ppm PCB and either 100 or 50 ppm α BHC or 250 or 100 ppm β BHC. Thus PCBs enhanced the tumor development of liver tumors of α or β BHC isomers.

Rats

Dietary exposure of male Sherman strain rats to levels of 500 ppm Aroclor 1254 (36.4 mg/kg body weight) for 6 months resulted in pronounced lipid accumulation in the liver which persisted for a 10 months observation period following the discontinuation of

MONS 056346

the dietary exposure to PCBs. Adenofibrosis (cholangiofibrosis) which was observed in the livers of rats ingesting the PCB for 6 months was still present 10 months later. Whether this lesion is persistent could not definitely be determined since high levels of the higher chlorinated biphenyl isomers were still present in adipose tissue and liver (Kimbrough, et al., 1973). Adenofibrosis is a focal proliferation of glandular epithelium forming ducts often surrounded by extensive fibrosis. This lesion usually occurs concomitantly in rat livers with hepatocellular carcinomas and has been produced by many known hepatocarcinogens. It was first described by Edwards and White (1941). The extremely long retention of some PCB isomers in rats was again noted when male Sherman strain rats were fed 100 ppm Aroclor 1254 for 6 months and then allowed to recover for 16 months. 4.4 ppm of PCB derived material was present in the liver and 152 ppm in adipose tissue on a wet weight basis (Kimbrough, 1976).

Continued dietary exposure to commercial PCB mixtures in mammals results in their storage in adipose tissue and over an extended period high levels may be attained (Curley, et al., 1971; Burse, et al., 1974). Blood levels are more a function of the dose, the rate of metabolism, availability of binding sites in blood, chemical characteristics of the compound given, rather than the amount stored in adipose tissue (Smrek, et al., in preparation).

MONS 056347

In another study, 200 experimental female Sherman strain rats were fed 100 mg/kg Aroclor 1260 in the diet (11.6 - 4.3 mg/kg body weight) for approximately 21 months and 200 female rats were kept as controls. A total of 184 experimental rats and 173 controls survived to sacrifice at 23 months. Hepatocellular carcinomas were observed in 26/184 experimental rats and in 1/173 controls. None of the controls but 146/184 experimental rats had neoplastic nodules within the liver (hyperplastic nodules). Areas of hepatocellular alteration were noted in 28/173 controls and 182/184 experimental rats. The incidence of tumors in other organs was not altered by PCB ingestion and metatases from the liver tumors were not observed (Kimbrough et al., 1975). Adenofibrosis of the liver was also noted in this study. In reporting these liver tumors the classification developed in a rat liver tumor workshop was followed (Squire and LeVitt, 1975).

Liver tumors (multiple adenomatous nodules) were also induced in 6/10 female Donryu rats with Kanechlor 400 but not in male rats. The dietary exposure varied throughout the study from 38-616 ppm (Kimura and Baba, 1973). The induction of liver tumors by known carcinogens in Sprague Dawley rats such as 3'methyl-4-dimethylamino-azobenzene, N-2-fluorenyl acetamide and diethylnitrosamine was inhibited by Kanechlor 500 (Makiura et al.,1974).

Groups of 50 male and female Charles River rats were fed dietary levels of 0, 1, 10 and 100 ppm of Aroclor 1242, 1254, and 1260 respectively (Calandra, 1976). At 3, 6, and 12 months after

MONS 056348

onset of the experiment, 5 rats per each group and sex were sacrificed. The surviving rats were sacrificied 24 months after onset of the experiment. The only body weight depression noted was observed in the female rats fed Aroclor 1254. The author reported that important histopathologic changes were only noted in the rats fed the different Aroclors for 24 months.

The liver weights were increased in the male and female rats fed 100 ppm of Aroclor 1260 and 1254 and only in females fed Aroclor 1242 at a dietary level of 100 ppm. It was noted that of a total of 20 surviving male and female rats (10 male and 10 female rats) fed 100 ppm Aroclor 1242 in the diet for 24 months 8/20 rats showed liver lesions called nodular hyperplasia, 2/20 had hepatomas and 1/20 had a cholangiohepatoma.

A total of 27 male and female rats (13 male and 14 female rats) survived to 24 months in the groups of rats fed 100 ppm Aroclor 1254 of these 13/27 had nodular hyperplasia of the liver 4/27 showed hepatomas and 2/27 showed cholangiohepatomas.

Similar findings were made in the total 27 surviving male and female rats fed 100 ppm Aroclor 1260 for 24 months. In these groups 7/27 male and female rats had nodular hyperplasia of the liver. 5/27 had hepatomas, and 2/27 had cholangiohepatomas.

Groups of 4 male and 4 female dogs were fed dietary levels of 0, 1, 10, and 100 ppm Aroclor 1242, 1254, and 1260 respectively for 2 years, 1 female fed 100 ppm Aroclor 1242, 1 male fed 10 ppm Aroclor 1242, 1 female dog fed 100 ppm Aroclor 1254, 1 female

MONS 056349

fed 10 ppm and 1 male and 1 female fed 100 ppm Aroclor 1260 died. At the dietary level of 100 ppm of either Aroclor 1254 or Aroclor 1260 the body weight gain was slightly decreased for both sexes. Aroclor 1260 also reduced the body weight gain in the females at the dietary level of 10 ppm. In the beagles fed Aroclor 1260 at a dietary level of 100 ppm, the liver weight was increased and the serum alkaline phosphatase was elevated. All but 1 dog that died had either chronic peritionitis or chronic pneumonitis. Many of the dogs fed 10 or 100 ppm of different PCBs showed chronic inflammation of a variety of organs. Male dogs fed Aroclor 1254 showed diffuse hyperplasia of the interstitial cells of the testes and aspermatogenesis (Monsanto, 1971).

Decachlorobiphenyl

No long term studies are available.

Polybrominated Biphenyls

No long term studies are available.

Chlorinated Dibenzofurans and Chlorinated Naphthalenes

No long term studies are available.

Immunosuppressive Effects of PCB and PBB

Polychlorinated biphenyls (PCB) have been implicated as immunosuppressants. Friend and Trainer (1970) found a decreased resistance to duck hepatitis virus in PCB exposed ducklings.

MONS 056350

PCB has been shown to produce lymphoid atrophy in rabbits (Street and Sharma, 1975; Vos and Beems, 1971), chickens (Flick et al., 1975; Vos and Koeman, 1970) and guinea pigs (Vos and DeRoij, 1972; Vos and van Genderen, 1973). In addition, PCBs were found to suppress humoral immune responses to several antigens in rabbits (Koller and Thigpen, 1973, Street and Sharma, 1975) and guinea pigs (Vos and DeRoij, 1972, Vos and van Genderen, 1973) and to suppress cell mediated immune reactions in guinea pigs (Vos and van Genderen, 1973). In all of these studies the PCBs studied have been commercial mixtures; the role of contaminants such as chlorinated dibenzofurans needs to be considered.

The effect of HBB on the immune system was studied in chickens and guinea pigs (Vos and van Genderen). In chickens the atrophy of the lymphoid tissue was observed in bursal follicles and in the spleen. It suppressed the humeral immune response in guinea pigs.

MUTAGENICITY AND TERATOLOGY

Polychlorinated Biphenyls

The mutagenicity and cytotoxicity of PCBs, Aroclor 1242 and 1254, were studied by Green et al., 1975, a, b. The lack of mutagenic activity as measured by the dominant lethal test was reported for male Osborne-Mendel rats subjected to four different dosage regiment: (1) single oral intubations of 625, 1250 or 2500 mg/kg (for Aroclor 1242); (2) five daily doses of 125 or 250 mg/kg (for Aroclor 1242), 75, 150 or 300 mg/kg (for Aroclor

MONS 056351

1254); (3) five daily doses of Aroclor 1254 at 150 mg/kg, then starved overnight prior to mating; (4) chronic feeding for 70 days at a dietary level of 25 or 100 ppm prior to mating (Green et al., 1975a). In a second study, bone marrow and spermatogonial cells were cytogenetically investigated from Aroclor-treated Osborne-Mendel rats. Aroclor 1242 was given orally at single doses of 1250, 2500, or 5000 mg/kg or as four daily doses of 500 mg/kg/day; Aroclor 1254 was administered orally as five daily doses of 75, 150, or 300 mg/kg/day. No statistically significant increases in chromosomal aberations were found for the Aroclor-treated rats. As indicated by Green et al, (1975 b), while these studies were negative in regard to chromosomal mutations, they do not entirely rule out the possibility that PCBs may induce point mutations. To date, there are no known reports in the literature concerning the induction of point mutations by PCBs in laboratory model systems. The observations of Nilsson and Ravel, 1974, in Drosophila where no chromosomal breakage was found among Clophen A30- or A50-treated flies, are fully consistent with those of Green et al., (1975, a, b).

TERATOLOGY

Female NMRI mice, orally administered 0.025 mg/day of Clophen 60, showed evidence of an increased estrus cycle from 6.6 $\pm$ 2.5 days to 8.7 $\pm$ 4.3 days and a decreased ova implantation rate from 87.0 to 79.5% (Orberg and Kihlström, 1973). Kihlström et al., (1974 a), also demonstratad a decrease in the frequency of implanted ova

MONS 056352

among young animals nursed on milk containing PCB (Clophen 60). Both the male and the female contributed to the magnitude of the reduction of implantations. The mechanism by which exposure of the developing male to milk containing PCB leads to a reduction in the precent of ova implanted is unknown but need not necessarily involve dominant lethal events.

No reports of frank terata were found, but a number of studies indicated the facility of PCBs for placental transport. Grant et al., (1971 b), showed that the concentration of PCB in fetal tissue from oral administration of Aroclor 1254 was dose-dependent and that accumulation occurred in fetal liver where higher concentrations were found than in maternal liver. Placental transport of PCB has been reported for the rabbit, rat, mouse, and cow (Grant et al., 1971; Platonow and Chen, 1972; Berlin et al., 1975; Melvas and Brandt, 1973; Brandt and Ullberg, 1973). Evidence of placental transfer was also observed among Yusho patients. While PCB has no known or clearly defined teratogenic effect in mammals, their easy passage across the placenta suggests the possibility of potential for some form of fetal toxicity.

Chlorinated Dibenzofurans

No information available.

Brominated Biphenyls

15 pregnant rats were force fed 100 mg hexabromobiphenyl/kg

MONS 056353

body weight on days 6, 8, 10, 12, 14 and 16 of pregnancy in corn oil. 14 control rats were given corn oil. The pups were obtained by cesarian on day 19 of pregnancy. No terata were observed and no difference between control and experimental rats was noted in any of the other parameters studied.

5 control and 6 PBB treated animals were treated with colchicine and bone marrow was studied cytogenetically. Animals treated with PBB and colchicine had higher metaphase and mitotic indices than non-treated animals. Chromosome aberrations were not noted (Fiscor and Werts, 1976).

Toxicity of Chlorinated Naphthalenes

The following information is derived from a review published by the EPA, namely Environmental Hazard Assessment Report 'Chlorinated Naphthalenes', EPA-560/8-75-001*. For literature references please refer to this report.

Animal Studies

The following tables summarize the available studies in laboratory animals. Three routes of exposure are reported.

*This document is available through the National Technical Information Service, Springfield, VA 22151. 'Preliminary Environmental Hazard Assessment of Chlorinated Naphthalenes, Silicones, Fluorocarbons, Benzenepolycarboxylates and Chlorophenols'. (NTIS accession number PB-238-074/AS)

MONS 056354

TABLE 9

(a) Dietary Exposure

Compound	Species	Dose Level	Days Exposed	Effect Reported
Mono-		None reported		
Di-	Rat	5g/Kg feed	15	Increase in liver weight, growth impaired, rough coat.
Tri-	Mouse	2.5 mg/day	20	None.
Tri-	Rat	300 mg/day	9-136	Fatty infiltration of liver.
Tri/Tetra	Rabbit	15 mg/Kg body wt.	60	None.
Penta	Rat	50 mg day	63	Jaundice/fatty degeneration of liver.
Penta/Hexa-	Rat	300 mg/day	33	Death with yellow livers and extreme fatty degeneration.
Penta/Hexa	Rat	100 mg/day	55	Death with yellow livers and extreme fatty degeneration.
Penta-	Guinea pig	2.5 mg/Kg in oil	48	Death-Severe weight loss fatty degeneration of liver.
Hexa-	Rat	20 mg/Kg	84	Weight loss.
Hexa-	Rat	63 mg/Kg	84	Weight loss.
Hexa-	Rat	200 mg/Kg	84	Death.
Heptachloro-		None reported		
Octa-	Rabbit	1 g	Single dose	Death in seven days.
Octa	Rat	0.5, 2 or 5g/Kg	22	Decrease in liver but not plasma Vit. A.

MONS 056355

TABLE 10

(b) Inhalation Studies

Compound	Species	Dose Level	Days Exposed	Effect Reported
Tri-	Rat	0.05-0.2 mg/L	2 hr/day for 20 days	No toxic effect.
Tri-	Rat	1.31 mg/L	16hr/day for 134 days	No toxic effect.
Tri-	Rat	10.98 mg/L	16hr/day for 102 days	Slight liver discoloration, 5% rats showed increased fatty degeneration
Penta-	Rat	1.16 mg/L	16hr/day for 52 days	Jaundice, enlarged liver and 69% fatality.

No studies have been reported for the other chlorinated naphthalenes.

(c) Dermal Studies

Compound	Species	Conc Applied	Days of Application	Effect
Mono-	Rabbit(ear)	90 mg/g Acetone	5-7	Mild reddening.
Mono-	Rabbit(ear)	500 mg/g Acetone	5-7	Severe reddening, no decrease of sebaceous glands.
Tri-	Rat(skin)	Not given	2 hr/day/40-60 days	None reported
Tri-	Mice (skin)	Not given	2 hr/day/40-60 days	None reported
Penta-	Swine (skin)	60 mg/Lx3L	6 days/week-4 weeks	Slight hyperkeratosis.
Penta-	Rabbit(ear)	30 mg/g acetone/day	5	Mild dermatitis, hair folicular attenuation.
Hexa-	Rabbit(ear)	30 mg/g acetone	5	Decrease in sebaceous glands

MONS 056356

TOXICITY IN BIRDS

Turkey Poults

Penta/Hexachloronaphthalenes in the diet.

(a) 5 ppm for 40 days caused 23% reduction in weight gain with 6.5% mortality.

(b) 100 ppm - all poults died within 33 days. Histopathologic examination showed enlarged and darkened livers.

The LC_{50} was 20 ppm with an average decrease in weight of 51%.

Chickens

Penta/Hexachloronapthalene in the diet.

(a) No effects reported at dietary levels of 4 and 20 ppm (35 days).

(b) 100 ppm prevented egg production (35 days).

(c) 2550 ppm caused 100% mortality (14 days).

Effects reported included enlarged fibrous livers, lack of feather pigmentation and pericardial and peritoneal edema. A fourfold increase in Vitamin A in the diet decreased toxic effects.

MONS 056357

TOXICITY IN DOMESTIC ANIMALS

Ingestion is the most important route for exposure of domestic animals.

Cattle

Highly chlorinated naphthalene poisoning in cattle is known as bovine hyperkeratosis or X-disease. Detailed information on toxic doses is lacking. Penta/hexa mixture at a dose level of 5.5 mg/Kg body wt (orally) for 5 days caused a sharp drop in plasma Vit. A by the end of the third day and depressed plasma Vit. A for over 30 days. A single oral dose of 1 mg/Kg body wt of hexachloronaphthalene caused mortality within two weeks. The first effect of chloronaphthalene poisoning is an interference with the biotransformation of carotene to Vit. A. The Vit. A. deficiency is quickly followed by inflammation of the oral mucosa, lacrimation, excessive salivation and irregular food consumption. Gross physical effects that develop during the course of disease include a general thickening of the skin caused by over development of the skin's hairy layer with loss of hair (hyperkeratosis). There may be degeneration or irregular growth of the horns. Continued exposure results in anemia, dehydration, loss of weight, fever and death. There may be severe liver damage.

Swine

No toxic effects were observed at levels of 100 mg/Kg

MONS 056358

body wt of the chlorinated naphthalenes (degree of chlorination was not stated). 150 mg/kg body wt caused a marked depression of plasma Vit. A, and death occurred at a dose level of 198 mg/Kg body wt.

Sheep

Toxic effects are observed at doses of chlorinated napthalene 10 X that required to produce toxicity in cattle. Sheep do not show cutaneous hyperkeratosis or an excessive drop in plasma Vit. A as observed in cattle.

Effects reported include nasal discharge, weakness, loss of weight, loss of appetite, ascites, necrosis and cirrohsis of the liver and cardiovascular effects.

Toxicity in Man

The most important route for mans occupational exposure to chlorinated naphthalene is via the inhalation route. Dermal absorption route may also occur.

Penta/hexachloronaphthalene at a concentration of 30 mg/g acetone, applied to the backs of human volunteers for a period of six weeks caused typical chloracne.

Daily topical application of mono-dichlorinated naphthalene to the human ear in mineral oil suspension for 30 days caused no observable effect. Two clinically distinct but oftern concurrent syndromes have been described, namely, liver necrosis, and chloracne

MONS 056359

with itching. A number of deaths involving acute atrophy of the liver have been reported; with the course of the disease similar to other forms of severe liver damage. Autopsy of fatally exposed workers show severe yellow atrophy of the liver. Definite data relating to dose response effects are in general lacking. However, two dermal studies with man have been reported.

SUMMARY

The degree of toxicity of chlorinated napthalenes increases with the degree of chlorination. The higher chlorinated naphthalenes (especially hexa and penta) have been associated with chloracne and liver damage in man. Dose response effects have not been established.

Cattle are more susceptible to the effects of chlorinated naphthalene than other domestic animals. The effects observed include development of Vit. A deficiency, hyperkeratosis and liver damage.

MONS 056360

HUMAN EXPOSURE TO POLYCHLORINATED BIPHENYLS

I. Introduction

Although the polychlorinated biphenyls (PCBs) have been used in a wide variety of industrial applications in the U.S. from 1930 until 1970, when their distribution was voluntarily restricted to closed systems, there is scant information on human exposure to adverse human health effects in the U.S. In the U.S., minimal human exposure of the population to PCBs is limited to food, air and water, while significant human exposure appears to be limited to sports fishermen consuming fresh water fish from contaminated streams and lakes; and to occupational exposure in industrial workers.

II. Dietary Exposure to PCBs

The May 1972 Report of the Interdepartmental Task Force on PCBs summarized the findings in food sampling programs conducted by the Food and Drug Administration and the Department of Agriculture. Jelinek et al., (1975), have provided an update to that summary and evaluated trends which is presented below.

The 1972 Task Force report outlined three broad sources of food contamination. These are restated here in order to show which sources have been effectively controlled and which sources apparently persist, as reflected by results obtained in continued food sampling.

MONS 056361

1. Environmental contamination - background levels of PCBs in fish from lakes and streams.

2. Industrial accidents - isolated incidents involving direct leakage and spillage or contact of PCB fluids and other PCB containing materials on animal feeds, feed ingredients or food.

3. Food packaging materials - PCB migration to foods packaged in PCB contaminated paper products.

As discussed in the 1972 Task Force report, there were a number of other positive findings in foods which could not be traced to one of these broad sources prior to 1972 and the source of such occasional findings remain speculative. However, in more recent years, essentially all positive findings of PCBs in the food supply have been attributable to one or more of these three sources.

Jelinek and Corneliussen (1975) reported that for the 1969-1975 period, there has been a significant decrease in PCB levels in all foods with the exception of fish, where no particular trend has been noted. From that observation they concluded that procedures instituted to exclude the use of PCBs in "open" applications have been effective, but that more needs to be done to prevent their entry into the acquatic environment.

Jelinek and Corneliussen (1975) presented data on FDA's sampling program showing a very conclusive decline in the FYs 1973-1975

MONS 056362

period in terms of occurrence rate of PCBs and maximum levels found in milk, eggs, cheese, animal feeds and components, processed fruits, and baby foods. Except for fish, occurrence rates in FY'75 had dropped to zero or less than 1% for all categories, and there were no findings in excess of the existing temporary tolerances. Similar data were presented covering USDA's sampling of meat and poultry which also showed a decline, to 0.3% positive and only 0.6% above the temporary tolerance.

Jelinek and Corneliussen (1975) also reviewed FDA Total Diet Study findings (FY'71-75) which showed that all food classes of the total diet have declined to no PCB occurrence and no calculable daily intake of PCBs, except in the meat-fish-poultry composites. About 40% of these composites continue to contain detectable PCBs, although only traces have been detected in the latter years. The limit of quantitative detection in the Total Diet Study is about 0.05 ppm; positive findings below that level are generally reported as traces since they are not reliably quantitated.

Occurrence of PCBs ended in 1973 in all other categories, notably in dairy product composites and the composites of grain and cereal products. This probably reflects the control measures effected on animal feeds and on the control of ingredients in recycled paper used for food packaging. What remains, based on the total diet study, is a continued low level occurrence (about 40% positive and only at trace levels) in the meat-fish-poultry

MONS 056363

composites. The fact that levels in these composites have declined to only traces further supports the inference that the meat and poultry and eggs no longer contain detectable PCBs and that the low level findings are primarily due to the fish in those composites. This would imply that the PCB levels in the diet may have "bottomed out" and may remain static until such time as there is a change in the levels for fish.

Estimates of dietary intake of PCBs based on the Total Diet Study must be taken as gross estimates because only traces are currently found and some type of estimated value must be assigned to those traces to calculate microgram per day intakes of PCBs. Jelinek and Corneliussen used a set of consistent assumptions for those traces in computing the daily intakes for the FY-1971-75 period. Their calculations showed a steady decline from 15 mcg per day down to 8.7 mcg/day, with all of this coming from the meat-fish-poultry group in FY'74-75 (minor and declining portions attributable to other food classes in earlier years.)

Review of this information implies that, although the various assumptions for trace residue reportings and varying food consumption data have a significant effect on daily intake calculations, an overall estimate in the order of 5-10 mcg/day PCB dietary intake of PCBs for th general public seems reasonable. This figure would not be applicable to diets which may include regular consumption of fish from certain locations with high PCB levels.

MONS 056364

III. PCB Residues in Fish

Review of the data gathered by various agencies on fish residues indicates a serious incompatability of sampling programs for obtaining data which reliably define trends in PCB levels. The Food and Drug Administration's primary concern is the levels which exist in the edible portion of commercially imported fish which are marketed interstate. Therefore, in most cases, the heads, entrails, skin and fins are excluded. Furthermore, FDA sampling has not included fish which are caught and consumed locally (freshwater fish) and the sampling has dealt with the most important consumption species which are primarily of marine origin and which generally do not contain PCB levels as high as freshwater species from certain locations.

In order to determine the human exposure to PCBs through dietary fish, one must know the levels of PCBs residues in the edible portions of fish, and the amounts of the various kinds of fish consumed by the population at large, and by special subpopulations. During the past few weeks, information has been compiled by National Marine Fisheries Service-NOAA on the PCBs levels of these residues in marine and freshwater fish and shellfish, and on dietary habits.

Table 11 lists the 20 most important types of fish eaten in the U.S. today, and the mean daily amount of each type eaten by

MONS 056365

TABLE 11

Fish and Shellfish Consumption in the United States
(September 1973-August 1974)

	Rank	Amount (mill.) lbs/yr)	Percent of total by wt.	Number of Actual Users (millions)	Mean amount per user (grams/day
Total		2957.	100.	197.	18.7
Tuna (mainly canned)	1	634	21.4	130.	6.1
Unclassified* (mainly breaded, incl. fish sticks)	2	542.	18.4	68.	10.0
Shrimp	3	301.	10.2	45.	8.3
Ocean Perch*	4	149.	5.0	19.	9.7
Flounder	5	144.	4.9	21.	8.6
Clams	6	113.	3.8	18.	7.6
Crabs/Lobsters	7	110.	3.7	13.	10.6
Salmon	8	101.	3.4	19.	6.7
Oysters/Scallops	9	88.	3.0	14.	7.8
Trout**	9	88.	3.0	9.	12.3
Cod*	11	78.	2.7	12.	8.1
Bass**	12	73.	2.5	7.6	12.0
Catfish**	12	73.	2.5	7.5	12.1
Haddock*	12	73.	2.5	11.	8.6
Pollock*	15	60.	2.0	11.	6.8
Herring/Smelt/ Sardines	16	54.	1.8	10.	6.7
Pike**	17	35.	1.2	2.5	17.4
Halibut*	18	32.	1.1	5.0	8.0
Snapper	18	32.	1.1	4.3	9.3
Whiting	20	25.	0.9	3.2	9.7
All other classified		152.	5.1	n.a.	n.a.

* Mainly imports
** Fresh water

MONS 056366

the subpopulations of actual users. The Table includes all dietary fish, from sport fishing and from domestic and imported commercial fishing. These 20 items comprise 95% of the fish products eaten. The results are taken from information in a recent Seafood Consumption Study by the National Purchase Diary of Schaumburg, Illinois, sponsored by the Tuna Research Foundation, and National Marine Fisheries Service data on the amount of fish and shellfish processed and distributed in the U.S. Several items in the Table are worth noting for purposes of the present analysis. (1) Although ninety-three percent of the U.S. population (197 million) eat fish, the average annual per capital consumption of fish is small: 15.0 lbs/year--fish eater; (2) a large "Unclassified" fish fraction exists in the U.S. diet, ranking just below tuna in importance; (3) the major portion of many of our most familiar types of seafood is imported; and (4) freshwater species, led by trout, bass, and catfish, comprise about 9% of our total fish diet.

A compilation of PCB Data, representing the results of all the measurements known to NMFS of PCBs in fish used in the U.S. diet is presented in the Compendium of PCB data. It includes data from government reports, and state and private publications. An effort was made to include current, unpublished data from marine laboratories. The compilation includes the results of a comprehensive literature search by the NOAA-Environmental Data Service (EDS). An EDS-ENDEX data search for unpublished data

MONS 056367

and a EDS-OASIS search were made, crossing marine food species with PCBs.

Two significant sources of data--the 1975 FDA-Market Basket results, and the results of the extensive Fall 1975 New York State Department of Environment analyses were not available to NMFS at the time Compendium was published. However, FDA has indicated that the 1975 Market Basket results do not differ dramatically from 1973/4 data. Preliminary results of the New York Study are included in Table 13.

Table 12 summarizes the PCB information assembled in the Compendium relating to the 20 most important kinds of dietary fish identified in Table 11. Although at one time or another, some PCB measurements have been made on many fish, Table 11 points out the inadequacy of information on PCB residues in the most important items in the fish diet. Sampling and analysis have been sporadic and not designed to monitor trends in human exposure. Systematic surveys of neither the important items nor the species most likely to be contaminated have been undertaken. Very few recent (1975) measurements have been reported.

However fragmentary the data on PCB levels may be, they do not include a single measurement exceeding 1 ppm in any of the ten most important fish foods, except for Dover sole and crab taken in the immediate vicinity of Los Angeles sewerage outfalls, and crab living on contaminated sediments in Upper Chesapeake Bay.

MONS 056368

TABLE 12

Maximum Reported PCB Residue Levels in
Fish Muscle (White Meat)

Rank#	Kind	Range and Max. Level Reported (ppm) (see Compendium)	Mean Level in the U.S. Diet
1.	Tuna	0-0.49 (1973)	Unknown*
2.	Unclassified (mainly breaded)	no data	Unknown
3.	Shrimp	0-0.167 (1971)	Unknown*
4.	Ocean Perch	0-0.25 (1974)	Unknown*
5.	Flounder/Sole	0.6.3[a] (1972)	Unknown*
6.	Clams	0-0.819 (1974)	Unknown*
7.	Crabs/Lobster	0-4.9[b] (1972)	Unknown*
8.	Salmon	0-0.5 (1974)	Unknown*
9.	Oysters, Scallops	0-0.43 (1974)	Unknown*
10.	Trout (excl. Lake Trout)	0-0.56 (1972)	Unknown*
11.	Cod	0-0.4 (1974)	Unknown*
12.	Bass	0.3-8.4[c] (1974)	Unknown*
13.	Catfish	0-4.4[d] (1974)	Unknown*
14.	Haddock	0-1.16 (1974)	Unknown*
15.	Pollock	0.0 (1974)	Unknown*
16.	Herring/Smelt/ Sardines (marine	0-1.6 (1974)	Unknown*
17.	Pike	0-0.65 (1974)	Unknown*
18.	Halibut	0-0.149 (1974)	Unknown*
19.	Snapper	0-0.1 (1974)	Unknown*
20.	Whiting	0.0 (1974)	Unknown*

These 20 items compose 95% of the fish in the U.S. diet.

* Probably well below 1 ppm

[a]Single Dover Sole taken near Los Angeles municipal sewerage outfall. The mean value of Sole samples taken in this vicinity was 1.3 ppm in 1975. Mean values of other samples taken away from outfalls were less than 1 ppm in 1975. In any case, although Dover sole is an important food fish in Northern California, it is not taken commercially in Southern California waters.

[b]Single yellowneck crab taken near Los Angeles outfall-mean of group including this crab was less than 1 ppm. A composite of Upper Chesapeake Bay crab contained 1.2 ppm in 1974.

[c]Taken in 1974 from St. Croix River, Wisconsin--mean of 4 White Bass was 6.2 ppm.

[d]Taken from Mississippi River, Minnesota--mean of a sample of 5 catfish was 2.5 ppm.

MONS 056369

No more than 1.6 ppm has been reported in items 11 through 20 in importance, with the exception of freshwater bass and catfish living in river and lake areas known to be contaminated with PCBs. Nonetheless, as emphasized in the Table, there have been no PCB measurements on the important unclassified dietary fish items, and the analytical information is in no instance systematic enough to permit an estimate of the actual mean PCB values, hence human exposure. The strongest statement that can be made, based on the incomplete data summarized in the Compendium, is that most of the important fish meat items eaten in the U.S. probably contain mean PCB residue levels well below 1 ppm.

Fragmentary evidence also leads to the conclusion that the PCB levels in fish liver, roe and oil are approximately ten times that in the edible muscle and the PCB concentrations are greatest in the older larger oily fish, especially those that spend part or all of their lives in contaminated lakes, rivers or estuaries.

Table 13 lists the location of the species where high PCB levels, exceeding the FDA 5 ppm guideline, have been reported. Although the trout, salmon and chub in Lakes Michigan and Ontario and the Hudson River bass, perch and eel constitute only a few percent at most of the national fish diet, in some areas they are a significant part of the fish diets.

In order to discover what is presently known about U.S. fish consumption habits, a wide range of government and private sources

MONS 056370

TABLE 13

Areas Where at Least One Example of High* PCB Residue Levels Have Been Reported in the Listed Species

Species	Location
Striped Bass	Hudson River, New Jersey
Chub	Lake Michigan
Carp	Mississippi River, Minn., Lake Onandaga, NY
Chain Pickerel, Alewife	Hudson River
Coho Salmon	Lake Michigan, Lake Ontario, Hudson River
Chinook Salmon	Lake Michigan, Lake Ontario, Hudson River
Steelhead Trout	Lake Michigan, Lake Ontario
Lake Trout	Lake Michigan, Lake Ontario, Lake George, NY
Bass, Smallmouth	Lake Onandaga, Genessee River, NY Hudson River, Mohawk River, St. Lawrence River
White Perch	Lake Onandaga, Hudson River
Alewife	Hudson River
White Sucker	Hudson River, Mohawk River
Walleye	Hudson River, Mohawk River, Black River, NY
Bass, Largemouth	Hudson River, Mohawk River
Yellow Perch	Hudson River
American Eel, Crabs, Sturgeon	Hudson River
Rock Bass, Catfish	Hudson River
Bluefish	Long Island Sound (muscle from one large oily fish contained 8 ppm)

This Table includes recent data from New York State Department of Environmental Conservation not included in the NMFS Compilation.

The Hudson River above Fort Edward, New York, is not contaminated with PCBs and the residue levels in fish are quite low.

*Above the current 5 ppm FDA Guideline.

MONS 056371

were consulted. A number of pre-1970 studies are available, including a 1969 NMFS Survey of Fish Purchases and a 1968-70 HEW Ten State Nutrition Survey. In addition, a few specialized reports have been published, including annual FDA Regional Basket Surveys that do not include fish eaten in restaurants and institutions or by sport fishermen, and surveys by the Sport Fishing Institute and a 1974 Texas A&M Analysis of Seafood Consumption Patterns in Texas. However, the most up to date and comprehensive data are available in the previously mentioned 1973-74 Seafood Consumption Study by the National Purchase Diary.

Table 14 summarizes results of fish consumption demographic information that defines the number of U.S. consumers who would exceed an arbitrarily set dietary micro-constituent level. To date, this kind of information is available only for total dietary fish and one subcomponent, tuna. Table 14 indicates that 75 million U.S. consumers eat more than the national average of 18.7 grams of fish per day and 29 million would exceed the present dietary intake limit of PCB (175 mcg/day) if the 35 or more g/day of fish eaten was contaminated on average to 5 ppm, the current FDA guideline level. There is, of course, no evidence that the mean PCB level in fish is anywhere near 5 ppm. Nonetheless, should it in the future reach 1 ppm, then by an extrapolation from Table 14, an estimate can be made that about 200,000 U.S. consumers would be exceeding the 200 mcg/day FDA limit. As

MONS 056372

TABLE 14

Number of U.S. Consumers Who Eat More Than X

X (g/day)	Number (thousands of consumers) Total Fish	Tuna
0	197,000	130,000
18.7 (national avg.)	75,000	5,700
35	29,000	1,300
50	14,000	400
100	2,000	43
150	400	10

* Derived from graphs on pp. 21 and 23 of the Seafood Consumption Study, September 1973--August 1974 by the National Purchase Diary, Shaumburg, Illinois, 1975. Sponsored by the Tuna Research Foundation.

MONS 056373

another hypothetical case, if the PCB level in tuna should ever reach 1 ppm, then about 5,000 tuna fish consumers, those who eat in excess of 175 g/day, would exceed the 200mcg/day limit from tuna alone.

Survey data do show that in general U.S. fish eaters include a wide variety of fish items in their diet. Thus, even if certain items contain toxic micro-constituents at or near FDA guideline level, the great majority of consumers would receive only a small fraction of their average dietary intake from these sources.

Clearly, there is a need for demographic information, similar to that available on total fish and tuna, on the other principal foodfish items in the U.S. diet; and a need to extend and better measure the high-consumption tail of the distributions, to get a correct picture of the number and type of consumers eating in excess of 100g/day of any single fish product.

IV. Conclusions

At present, it is difficult to estimate human exposure to PCB from eating fish, either for the population as a whole or for subgroups at higher risk. Fragmentary evidence suggests that the exposure to the population as a whole from PCB residues in ingested fish is probably well below 19 mcg/day-consumer, based on PCB levels which are probably well below 1 ppm and 19g fish consumed/day.

MONS 056374

Although it is important to know the total population exposure, the first priority should be given to defining the dietary intake of PCBs in special subpopulations at high risk by reason of high consumption of fatty fish, or high sensitivity to the toxic action of PCBs. Such populations might include pregnant women and young children, dieters, low income groups and sport fishermen living in coastal and lake regions where there is known PCB contamination. Primate studies suggest that pregnant women and young children will be particularly sensitive to PCBs, and dieters, and in some areas low income groups may rely heavily on fish for food. For each subpopulation, the amount and kind of fish in the diet and the PCB residue levels in the fish eaten must be obtained in order to determine exposure. None of the necessary information to define the human PCB exposure in subpopulations is presently known.

The first priority in systematically monitoring and analyzing for PCB residues in fish should therefore be among the oily fish such as salmon, trout, mackerel, shad, sardines, herring, bluefin tuna, sable fish, white fish, striped bass, and bluefish, that have been identified as significant food items in the diets of special subpopulations of consumers.

Although only minimal dietary exposure to PCBs may occur through food, there is evidence of potentially significant exposure

MONS 056375

in those sub-groups of the population who regularly consume freshwater fish from waters which are contaminated with PCBs at levels which exceed the FDA temporary tolerances. These sub-groups of the population would include sports fishermen and others who consume locally caught freshwater fish.

In surveys conducted by the State of New York and FDA, samples of 17 species of fish were collected from 10 points on the Hudson River between Glens Falls and Alpine, 13 samples were collected at Glens Falls and 68 samples below Glens Falls. The data from these surveys indicate that 53 out of 68 samples of fish collected at or below Fort Edward (approximately 7 miles below Glens Falls) contained PCBs in excess of 5 ppm. The average level of PCB contamination in these 68 samples was 31.3 ppm. In contrast, only trace levels were found in the 13 samples obtained at Glens Falls. A high proportion of finfish and eels from all sampling points below Glens Falls contained PCB levels in excess of 5 ppm. The Fort Edward sampling point had the highest levels for the species examined, with individual samples ranging from 20.1 to 178 ppm.

Twenty-eight of the 33 samples of coho and chinook salmon from Lake Ontario exceeded 5 ppm, with a range from 21 ppm to 24.6 ppm. Of the 4 samples of lake trout tested, results were in the range of 10 to 15 ppm. All other species of fish sampled from Lake Ontario were at 5 ppm or less PCBs.

The Great Lakes represent another area of the nation contaminated with PCBs. The Michigan Water Resources Commission has reported

MONS 056376

that many surface water samples contained PCBs at concentrations above the detection limit of 10 ppt. (10 significant levels of PCBs have been found in rivers and streams discharging into the Great Lakes. Effluents from wastewater treatment plants servicing industrialized communities have been found to be highly contaminated.

Sampling surveys of Great Lakes fish have shown that most species tested contained detectable levels of PCBs and that residue levels were generally proportional to fish size (age) and highest in the predator species. Except for whitefish, the species of commercial or sport interest (trouts and salmons) from Lake Michigan were found to be highly contaminated with PCBs. Data obtained from lake trout collected from various areas of Lake Michigan show mean PCB levels ranging from 3.06 ppm to 11.93 ppm.

The Michigan Department of Public Health has recently completed a study (Humphrey et al., 1976) which attempted to assess some of the consequences of human exposure to PCBs from the consumption of sportsfish caught in different areas of Lake Michigan. The study included exposed and control subjects from five areas of Michigan bordering on Lake Michigan. Exposed study subjects were those individuals who consumed at least 24 to 26 pounds of Great Lakes fish per year. Control subjects were those individuals who consumed less than six pounds of Great Lakes fish per year.

A preliminary assessment of the findings in the study indicate that the most frequently recorded quantity of fish consumed by the

MONS 056377

study participants was in the 24-25 pounds per year range. The highest recorded fish consumption over the two year period of the study was 180 pounds per year and the highest single season consumption was 260 pounds. Mean PCB levels in whole Lake Trout are reported as 18.93 ppm in 1973 and 22.91 ppm in 1974, and 12.17 ppm in Coho Salmon in 1973 and 10.45 ppm in 1974.

However, comparisons of PCB levels in raw vs. cooked fish indicate that actual human exposure to PCB from fish consumption is less than might be expected from the raw fish data. This is not unexpected since preparation (trimming away fatty tissue) and cooking have been shown to decrease the concentration of PCBs. For example, the PCB level in cooked Lake Trout consumed by the study participants ranged from 1.03 ppm to 4.67 ppm; in cooked salmon from 0.48 ppm to 5.38 ppm; and in other cooked fish from 0.36 ppm to 2.06 ppm. These levels are decidedly lower than the level of PCB contamination reported in raw trout and salmon.

PCBs were found in 501 blood and breast milk specimens collected from study participants during the study. The values ranged from a low of 0.007 ppm in blood in the control group to a high of 0.366 ppm in the exposed group. Although there was a wide range of blood values for each quantity of fish consumed, there was a highly significant correlation between the reported quantity of Lake Michigan fish consumed and the concentration of PCB in the blood of study participants. Using a natural log transformation

MONS 056378

of PCB values, the correlation between amount of fish consumed and PCB blood levels was significant ($t=6.24$, $p= 0.0001$), with higher reported fish consumption being associated with higher blood PCB levels.

No annual variation in PCB blood levels in humans could be demonstrated. The mean PCB blood values for the control and exposed groups did not appear to change markedly from 1973 to 1974. In addition, abstinence from Lake Michigan fish consumption for a period of 90 days or more did not change the PCB blood levels significantly. PCB blood levels over the abstaining period shows variation but no steady decline in PCB. In fact, more subjects showed no change or a rise than showed a decline in PCB blood levels over time.

The calculated quantity of PCB ingested by eating Lake Michigan fish averaged 46.5 mg/year and ranged from 14.17 to 114.31 mg/year. PCB ingestion for each individual was determined by proportioning the reported annual fish consumption by frequency of species eaten and the cooked fish PCB levels for those fish. The community average for cooked fish was used in instances were cooked fish determination were not available for a study participant. Because fish consumption was found to vary from year to year the average annual consumptic for each individual for the two baseline years of study was used in each case.

MONS 056379

Results from this study indicate the strong likelihood that the maximum allowable ingestion of 1 mcg/kg body weight/day recommended for protracted exposure to PCB is being exceeded by the majority of the exposed group participants, and by inference, by a substantial number of individuals participating in sport fishing and who consume quantities of contaminated fish. The calculated mean daily dose received by the exposed group is 1.7 mcg/kg/day and ranges from 0.49 mcg to 3.94 mcg/kg/day. If the average annual rate of PCB ingestion from fish indicated by these study results were continued over the years, and if net accumulation occurs, the average sports fisherman consuming contaminated fish could receive a total PCB dose equal to the 200 mg safety limit in approximately 4.3 years. Under the same set of assumptions, individuals consuming greatet than average amounts of contaminated fish would reach the total dose level sooner.

No adverse health effects or group of symptoms could be identified in the exposed group that were clearly related to PCB exposure. This implies that exposure to PCBs from eating contaminated fish at the levels observed and the presence of PCBs in these exposed persons has not caused any observable adverse health effects at the time of the study. However this does not exclude the possibility that effects too subtle for detection are occurring or the possibility of long-term adverse health effects.

MONS 056380

Considerable scientific interest has centered on the Yusho incident in Japan where in 1968 human intoxication with Kanechlor 400, a PCB manufactured in Japan, was noted when a heat exchange leaked this PCB into rice oil ("Yusho" oil) which was consumed by Japanese families.

The typical clinical findings included chloracne and increased pigmentation of the skin, increased eye discharge, transient visual disturbances, feeling of weakness, numbness in limbs, headaches and disturbances in liver function. Most of the babies born to mothers with Yusho had skin discoloration which slowly regressed as the children grew in size. Adult "Yusho" patients had protracted clinical disease with a slow regression of symptoms and signs, suggesting a slow metabolism and excretion of the PCB in humans, probably resulting from a long biological half-life.

A review of the literature extent in 1972 led to the following facts with respect to this tragic incident in Japan:

1. The average PCB content of the rice oil in the dose-response epidemiologic study was 2,500 ppm.

2. In this dose-response epidemiologic study, the average cumulative intake of PCBs, leading to overt symptomatology, was 2000 g. In this same study, the lowest dose leading to overt symptomatology was 500 mg.

MONS 056381

3. The toxicity seen was the result of the ingestion of the PCBs contaminant in the rice oil.

A summary updating of the "Yusho" incident was presented by Professor Kuratsune at the National Conference on Polychlorinated Biphenyls, Chicago, Illinois, November 19, 1975 (Kuratsune et al., 1976). This report affects the original conclusions enumerated above.

1. PCBs Content of "Yusho" Oil.

The first reported PCB determination was made on a canned contaminated rice oil produced on February 5, 1968, and whose consumption was associated with overt symptomology. One group of Yusho patients was associated with ingestion of this contaminated canned rice oil produced or shipped on February 5 and 6, 1968 (Kuratsune et al., 1972).

The detailed epidemiologic studies are given in the papers by Kuratsune et al., (1969) and Yoshimura (1971) and summarized in English by Kuratsune (1972), Kuratsune et al., (1971) and Kuratsune et al., (1972). Of 325 patients queried, 166 of 170 used canned rice oil produced or shipped on February 5 and 6, 1968, and 143 of 155 used bottled rice oil shipped from February 5 to 15, 1968. Tsukamoto et al., (1969) determined that the organic chlorine content of canned rice oils produced or shipped on February 5, 1968, was 1000 to 1500 ppm, by chemical and activation analysis. Since Kanechlor 400 had a 48 par

MONS 056382

cent chlorine content, if one attributed all the organic chlorine to this specific chlorinated biphenyl, then this canned rice oil contained, as an average, 2500 ppm PCBs. Kuratsune et al., (1969) reported that a qualitative GLC survey of 109 samples of bottled rice oil shipped between October, 1967, and October, 1968, showed "significant" PCBs content only in those samples between February 7 to 10, 1968. The largest amount was reported in the sample of February 7, 1968, but the quantity was not given. No bottled rice oil samples were available for February 5 and 6, 1968. However, in the paper by Kuratsune et al., (1971), the most marked contamination of bottled rice oil was for that shipped on February 7, 1968; and the maximum chlorine content was 462 ppm. One may assume that this number applies to the sample of bottled rice oil of February 7, 1968, giving this sample a PCBs content of 924 ppm. Kuratsune et al., (1976), reporting the work of Nagayama et al., (1975b), gives the PCBs analyses for three samples of toxic "Yusho" oil, used by three independent families with "Yusho", as approximately 800 to 1000 ppm. These were GLC/MS measurements. Nagayama, et al., (1975b), described these samples as follows:

"Rice oil used by patients with Yuso ("Yusho oil"): 3 samples of rice oil used by 3 independent families with Yusho in 1968. These samples had been kept in glass bottles with glass stoppers at room temperature in our laboratory until 1974 when the analysis was made."

MONS 056383

Kuratsune (1976) pointed out that these three samples were canned oil and considered to have been produced and shipped on February 5 or 6, 1968. This is also suggested in footnote a, Table 5, in the paper by Kuratsune et al., (1976). It was reported previously that, based on organic chlorine determinations, Tsukamoto et al., (1969) reported an average PCB content of the canned oil of February 5, 1968, to be 2500 ppm. Now, based on BLC/MS, we have a PCB content of ca 1000 ppm for canned oil sample of February 5 or 6, 1968.

What are the possible explanations for this discrepancy in values? One possibility is based on the assumptions associated with the method of measurement by Tsukamoto et al., (1969); namely, determination of total organic chlorine. Thus, any orgnic chlorine present in the rice oil, which was not a chlorinated biphenyl, would be included in the PCB analysis. Some possibilities include: chlorinated dibenzofurans, chlorinated naphthalenes, chlorinated paraffins, other chlorinated aliphatics and non-biphenyl aromatics.

Another possibility is related to the distribution, with time, of the contaminated rice oil samples. February 5, 1968, occurred on a Monday. The first rice oil samples reported to contain PCBs were produced on this date (Tsukamoto et al.; 1969). If the leak first occurred sometime over the weekend (February 3 and 4, 1968), and

MONS 056384

production was resumed Monday morning, February 5, 1968, then one would expect the samples of this date to be higher in PCB content.

Therefore, one would expect the first batches Monday morning to be highest with a dimunution of PCB content with time. Tsukamoto et al., (1969) reported the organic chlorine content of six canned oils produced Monday, February 5, 1968, as follows: 1020, 1170, 1070, 1080, 1030, and 1500 ppm. In the survey of bottled oils, Kuratsune et al., (1969) reported that for those samples where PCBs were found, the greatest amount was in the samples of February 7 (Wednesday); and after February 11. It was inferred from the paper by Kuratsune et al., (1971) that the PCB content of the bottled rice oil samples of February 7, 1968, was 924 ppm. In addition, in Table 5, Kuratsune et al., (1976) gave a value of 134 ppm PCB for a rice oil produced on February 10, 1968. Thus, the available data does confirm a diminution of PCB contamination of the rice oil with time. This is summarized below:

Date	Source	PCB content (ppm)	Method
Feb. 5, 1968	canned	2000-3000	organic Cl
Feb. 5 or 6, 1968	canned (?)	ca 1000	GLC/MS
Feb. 7, 1968	bottled	924	organic Cl
Feb. 10, 1968	bottled	134	?
After Feb. 11 1968	bottled	just detectable	GLC (?)

MONS 056385

The resolution of the varying PCB values for the canned oil cannot be made. However, Kuratsune (1976) confirmed that the production of rice oil had been suspended for an unknown period of time prior to February 6, 1968, when it resumed.

2. Dose-Response

There were 1291 "Yusho" patients as of April 30, 1975 Oema, 1975). Of this total group, the dose-response relationship was developed from a detailed analysis of 146 patients (Yoshimura 1971; Kuratsune et al., 1972). These 146 patients all consumed contaminated canned rice oil produced on February 5, 1968 contained 2000 to 3000 ppm PCBs as Kanechlor 400 (Tsukamota et al., (1972) estimated that the lowest dose producing symptoms was 0.5 gm.

If the PCB content of this canned oil was ca 1000 ppm, as discussed in the previous section, then the average dose producing an overt effect was 0.8 gm. and the lowest dose producing an overt effect was 0.2 gm.

3. Causative Factors.

Originally, rice oil contaminated with a heat exchanger, Kanechlor 400, a polychlorinated biphenyl, was associated with "Yusho" symptomatology (see discussion in section

MONS 056386

1 above). PCBs were identified in the contaminated rice oil consumed and in the blood and tissues of Yusho patients. Therefore, the effects seen were attributed to PCBs.

In the review by Kuratsune et al., (1976), a new factor was introduced into the system; namely, the canned rice oil was also contaminated with chlorinated dibenzofurans to the extent of 5 ppm. In addition, in this same paper by Kuratsune et al., (1976), they presented data of Bagayama et al., showing polychlorinated dibenzofurans in the liver and adipose tissue of Yusho patients, while none was found in that of a control group. The ratio of PCBs to PCDFs in Kanechlor 400, a Yusho oil of February 5 or 6, 1968, in adipose tissue and liver from a Yusho patient were 50,000; 200; 144; and 4 respectively. Thus, relative to Yusho oil, the liver with a PCB/PCDF ratio of 4 to 1, appears to selectively concentrate PCDFs relative to PCBs.

Relative to PCBs, PCDFs are about 250 times higher in the rice oil than in Kanechlor 400 (Nagayama et al, 1975b; Kuratsure et al., 1976).[1] They also stated that PCDFs may be a factor in Yusho disease. In another

[1]) It should be noted that we are comparing chlorinated dibenzofuran content of an unused Kanechlor 400 to that after prolonged use in a heat exchanger.

MONS 056387

paper Nagayama et al., (1975a) reported that it "is said that the toxicity of PCDF is said to be from 200 to 500 times that of PCB". At the lower factor (200), contaminated rice oil would be expected to be twice as toxic as Kanechlor 400. Also, in this same paper they stated that the symptoms seen in Yusho patients seemed to be more severe than would be expected just from the PCB intake associated with the oil.

If PCDF is 200 to 500 times more toxic than PCB (Nagayama at al., 1975a), then contaminated rice oil would be 2 to 3.5 times more toxic than that expected from its PCB content, alone. Kuratsune et al., (1972), estimated that if the average amount of contaminated rice oil ingested by a "Yusho" exposed person, is related solely in terms of its PCB equivalent, then the amount of oil needed to be ingested, on that basis, would be 1600 ml to 2800 ml. At a PCB content of 2500 ppm, this is an ingestion of 4 to 7 gm of PCBs equivalent; whereas at a PCB content of 1000 ppm, this is an ingestion of 1.6 to 2.8 gm of PCBs equivalent.

Conclusion

The complexities and uncertainties associated with the most recent reports of the "Yusho" incident in Japan, make it extremely difficult to quantify possible human health effects resulting from exposure to PCBs alone.

MONS 056388

V. Occupational Exposure to PCBs

The earliest reports of adverse health effects due to exposure of workers to PCBs in this country are probably those of Schwartz (1936) who described skin lesions and symptoms of systemic poisoning among workers who were said to have inhaled chloro diphenyls; their complaints included digestive disturbances, burning of the eyes, impotence, and hematuria. Patch tests with the chlorodiphenyls were negative, and Schwartz speculated that mechanical plugging of the follicles of the skin as the fumes solidified on it were responsible for the skin lesions; the chlorine present in the products were thought to then exert an irritating effect on the plugged follicles, and to thus cause suppuration. No quantitative data were reported, but a number of preventive practices were recommended.

There have been numerous reports over the ensuing years (Schwartz and Bartow, 1942), (Greenburg et al., 1939), (Drinkes et al., 1937), (Good et al., 1943), (Schwartz, 1943) and (Meigs, et al., 1954) of cutaneous eruptions and of systemic manifestations (sometimes fatal) as well, among marine electricians, machinists, capacitor and transformer manufacturing workers, and others occupationally exposed to PCBs. However, in many of these reports the exposures are described as having been to mixtures of chlorinated hydrocarbons, quite often of chlorinated naphthalenes and PCBs.

MONS 056389

The skin lesions described by Schwartz (1936) have come to be designated generally as "chloracne." Chloracne can be produced by a number of chemical compounds, including chlorinated dibenzofurans and certain isomers of the chlorinated dibenzodioxins (Kimbrough, 1972). Oily skin and large pores seem to predispose to the disease, while the opposite is the case for smooth, tender skin (Kimbrough, 1974). Chloracne also has occurred among workers engaged in the production of 2,4,5-T (Poland et al., 1971).

Part of the chloracne lesion resembles adolescent acne, but it is generally more severe, and lesion distribution is inconsistent with, although it may be superimposed upon, adolescent acne. It is known that chloracne can be produced by either the systemic absorption of chlorinated biphenyls or the direct application of chloracnegenic compounds to the skin. Systemic effects sometimes result after occupational chloracne has manifested itself; these may include loss of appetite, nausea, edema of the face and hands, abdominal pain, vomiting, and burning and soreness of the eyes. No fully satisfactory explanations have been made of the development of chloracne. Chloracne is generally very persistent, and there is the preferred control measure. An excellent review of this subject is that by Crow (1970).

The U.S. Occupational standards for PCBs are: 8-hour time-weighted average exposure limits of 1 mg/m^3 (skin) for the 42% chlorinated product, and 0.5 mg/m^3 (skin) for the 54% chlorinated product (CFR, Title 29, Part 1910.93). The National Institute for Occupational Safety and Health has under way the preparation of

MONS 056390

criteria for a recommended standard for occupational exposure to polychlorinated biphenyls. In addition to an environmental exposure limit, this document will provide comprehensive recommendations for medical surveillance, safe work practices, and engineering controls to ensure employee protection during occupational exposure over a working lifetime; it is scheduled for transmittal to the Department of Labor in late 1976 or early 1977. NIOSH also is undertaking certain studies of employee populations (mainly in U.S. capacitor factories) in order to better define the nature and extent of any chronic and/or life-shortening effects of exposure to PCB.

Practically all of the currently available information on worker health and PCB exposure is found in foreign literature. For example, Karppahen and Kolho (1974) reported on relationships between the concentration of PCB's in the blood of all, and in the adipose tissues of some, of 29 persons in "good health"* from

*Kolho has stated that the capacitor plant workers received quarterly health examinations, and, in addition to the clinical examination made at the time of the investigation, serum alkaline phosphatase, GOT, and GPT activities were determined; the 6 employees with the highest blood concentrations of PCBs also had BSP excretion tests performed. All results were normal.

In view of claims based on animal experiments that PCBs can induce liver microsomal enzyme activity, the authors also determined the half-time of antipyrin before and after phenobarbital induction (1 mg phenobarbital/kg body weight/day/3 days) for 6 capacitor plant employees and 6 controls; no enzyme induction was observed. Further, because of claims that PCB's have effects on steroid metabolism, 4 capacitor plant workers were tested for ACTH (serum, presumably); all results were reported to be within the normal range (letter from S. Hernberg to A. C. Kolbye, Jr., 12/23/75.

MONS 056391

three different employee groups in Finland. None of the employees had no history of occupational exposure to PCBs, six of them had handled PCB samples in an analytical laboratory, and the other eleven of them had been employed for 6 years in a capacitor factory where Aroclor 1242 was used as the impregnating fluid. It was stated that average PCB concentrations in the air of the capacitor factory had not exceeded "internationally accepted limits**," and that special attention had been given to skin protection.

Table 15 shows the observed tissue concentratins of PCB's; the authors were unable to detect any biological effects of the approximately 50-fold larger PCB concentrations in the blood of the capacitor plant employees, compared to the "unexposed" control group.

Ouw et al., (1974) conducted a survey to determine the degree of absorption and the health effects of exposure to "electrical grade" Aroclor 1242 (that "did not contain any impurities") for varying periods of time. PCB concentrations in the air of a capacitor factory in New South Wales, Australia*, were measured, and 34 occupationally exposed employees (15 males, 19 females, ranging in age from 33 to 55 years) were examined and compared with volunteer controls (23 males, 7 females ranging in age from 20 to 50

*The Joint ILO/WHO Committee on Occupational Health, in its sixth report (World Health Organization Technical Report Series No. 415, 1969), recommended for international adoption a "safe concentration zone" of 1 mg/m^3 for chlorinated derivatives of diphenyl.

**Aroclor 1242 is not manufactured in Australia (Ouw et al., 1974).

MONS 056392

TABLE 15

Concentration of PCB's

Subjects	In blood Fat basis mg/kg		In adipose tissue Fat basis mg/kg	
	Average	Range	Sample no	
Workers in capacitor factory	313	100-700	1	200
			2	11
			3	160
			4	285
			5	635
Persons handling PCB's in analytical laboratory	53	33-71	not analyzed	
Persons without any special exposure to PCB's	5.4	3.6-9.9	1	2.3
			2	1.5

From Karppanen and Kolho (1974)

MONS 056393

years) having no history of occupational exposure to PCB's. Study paramaters included occupational and medical histories, PCB-in-blood concentration estimates, and liver function (serum bilirubin, alkaline phosphatase, total protein, and GPT, and BSP excretion) tests.

The authors noted that exposed workers tended to complain of eye, face, and skin "burning," that the PCB "'fume' ... has a pungent smell which often causes persistent body odour," and that the employees with higher blood concentrations of PCBs complained most often of the skin lesions (one case of chloracne, five cases of "an eczematous rash on" the legs and hands), although there apparently was poor correlation of blood PCB's with the severity of the complaints. No significant health affects were observed among those of the 34 workers whose blood PCB concentrations were below 200 ppb.

There was a statistically significant difference between the blood PCB concentrations of the exposed group and the control group (P less than 0.01). Table 16 shows the concentrations of Aroclor 1242 in the capacitor plant air prior to and after exhaust ventilation system "improvements" had been made. (The Australian National Health and Medical Research Council recommended (Atmospheric Contaminants, 1970) exposure limit values of 1.00 mg/m^3 for PCBs of 42% and 54% chlorine content, respectively). Table 17 shows that there was no lowering of blood PCB concentration among those workers tested two months after the installation of a more

MONS 056394

TABLE 16

Arocloro 1242 concentrations in the air inside capacitor plant before and after improvement of exhaust ventilation system

No.	Areas in the Impregnation Room	Aroclor concentration in mg/m^3	
		Before	After
1	Area in the unloading tank in front of exhaust register from operator's breathing zone	1.44	0.75
2	Area in the unloading tank not in front of exhaust register	2.22	0.7
3	General atmosphere near tank	1.08	0.18
4	Soldering area	0.32	0.08

From Ouw et al (1974)

MONS 056395

Mean blood Aroclor 1242 levels before and after improvement of exhaust ventilation and the recommendation to use "suitable impervious" gloves.

Group	Mean blood Aroclor levels in ppb. Retention times relative to Aldrin I.		
	0.69	1.31	1.41
Before	281.6	135.1	58.41
After	477.2	225.4	524.7
Statistical differences	Not significant difference (P 0.01)	Not significant difference () 0.01)	Significant difference (P 0.05)

From Ouw et al (1974)

MONS 056396

efficient exhaust ventilation system and the concomitant recommendation to wear "suitable impervious gloves" in order to reduce PCB absorption through the skin. The authors suggested that a failure to adhere strictly to the glove recommendation might explain the continued elevation blood PCB levels. Another possibility might be that PCBs were continually mobilized from storage in adipose tissues.

The Japanese Ministry of Labor (Regulation for the Prevention of Disturbances due to Specified Chemical Substances, under the Act for Safety and Health of Workers, of April 28, 1971) established an occupational environmental exposure limit for PCBs of 0.5 mg/m^3 at 25° C, one atm (Ad Hoc Committee, 1974). Japanese importation of PCBs commenced around 1950, and early uses were as dielectrics in capacitors and transformers. In 1954, Kanegafuchi Chemical Industry Co., Ltd. started PCB production in Japan and it was at about this time that chloracne eruptions among workers were first reported. Slightly earlier (1953) incidents, however, were reported by Hara (1969), of changes attributable to PCBs in blood and urine findings among capacitor factory workers.

According to an Ad Hoc Committee sponsored by the Japanese Environmental Agency (1974), PCB concentrations in the air of a Japanese capacitor factory (where the major PCB constituent was "diphenyl pentachloride") were found to range from 0.37 to 6.75 mg/m^3; this was between the years 1953 and 1957, and prior to the

MONS 056397

date when the aforementioned occupational exposure limit was promulgated. The highest concentration was measured where PCBs were heated to drive out entrained/dissolved air. Admittedly, the analytical methodology for PCBs in those years was not as refined as that now in use, but it is thought likely (authors) that some employees were exposed continually at concentrations of several milligrams per cubic meter.

The Japanese literature is said to have contained no further reports on PCB-in-air measurements until the spring of 1972, just prior to the suspension of their usage. The production of PCBs was discontinued in Japan in June 1972, and their importation was discontinued in the following September. At that time Hasegawa et al., (1973) reported PCB measurements in the air of one PCB (Kanechlors 200-600) production plant (0.005-0.02 ppm), four capacitor manufacturing plants (Kanechlor 300 used) (0.01-0.05 ppm), and one plant where PCBs (Kanechlor 300) were used as a heat exchange medium (0.002 ppm). Peak values of 0.17 ppm and 0.67 ppm, respectively, were found in a capacitor plant "air-riddance" process area and in a capacitor impregnating area where tank leakage had occurred. Estimated (presumably 8-hour workday) time-weighted average exposures of affected employees were 0.02-0.03 ppm. Airborne PCBs in the capacitor plants were reported to consist of 70-80% vapor for material corresponding to Kanechlor 200, and of particulates larger than 0.1 micron for Kanechlor 300. The authors attributed the 3/1 vapor/particulate mix to selective evaporation of low boiling components of Kanechlor 300.

MONS 056398

Although no measurements were available, Hasegawa et al., (1973) estimated that environmental conditions in carbonless copy paper manufacturing facilities (using Kanechlor 300) were roughly similar to those found in capacitor factories. Beginning in February 1971, PCBs were phased out as the microcapsular solvent in this process, being replaced by "SAS" or KMC-oil;" however, during the period from November 1972 to January 1973, when occupational health surveys were being performed relative to SAS or KMC-oil toxicity, PCB concentrations of 0.013-0.4 ppb were measured in the environments of these facilities. General environmental concentrations of PCBs at that time were less than 0.0005 ppb, and outdoor PCB concentrations around the facilities were 0.0043 ppb (Hasegawa et al., 1973) and 0.009 ppb (Sato and Hasegawa, 1974). In an office room where carbonless copy paper was used, an air concentration 1.1 mcg PCB/m^3 was measured, and in the carbonless copy paper storage area of a post office, PCB concentrations of 8.7-21.1 mcg/m^3 were detected (Nishiyama et al., 1973).

The Japanese Ministry, in its "Regulations for Physical Examinations for the Prevention of Disturbances due to Specific Chemical Substances," promulgated on October 14, 1971, advised PCB users to examine employees for dermal andhepatic "symptoms" and their anamnesis, "subjective symptoms" such as anorexia and asthenia, and urine urobilinogen, in the first (pre-employment?) physical examination, and to "survey" working conditions, blood tests and liver function tests in the "secondary" (periodic re-?)

MONS 056399

examination. A 1973 notice adivsed those users of PCB's as heat exchange fluids to perform comprehensive health surveillance (Environmental Agency of Japan 1974). Yamamoto (1974) reported "positive" findings in 37 employees of 323 (total employment = 706 in 51 establishments) who were given "special" physical examinations in 1971.

Hara (1969) reported a 20-30% incidence of dermal "symptoms," consisting of "distinctive hair follicles" in exposed areas such as the face, neck, and forearm, and pimple-like skin eruptions of the face and neck, among employees in a capacitor factory that had been in operation between 1953 and 1963 (pentachlorobiphenyls) 1953-1957; Kanechlor 300, 1958-onward). No other, e.g., hepatic, dysfunction was observed, and no quantitative information was reported in the reference from which the preceding report was obtained; however, in a follow-up survey of workers in capacitor factories, Hara et al (1974) reported that by one year after the suspension of PCB usage skin findings had become milder.

Hasegawa et al., (1973) reported on a 1972 survey of capacitor factories, in which they noted that persons working in environments that contained 0.2-0.3 mg PCB's/m^3 (0.02-0.03 ppm) showed dermatolpgic ailments that included "brown chromodermatosis" of the dorsal joints of the hands and fingers and nail bed, and "acneform exanthema." Several cases of comedo or acneform exanthema of the jaw, back, and thighs were seen also. These signs were no longer observed one month after the cessation of the handling of PCB's.

MONS 056400

In a factory where Kanechlor 300 was employed as a heat exchange medium, the environmental concentration of PCB's was reported to be 0.02/mg/m^3, and no dermal manifestations were observed among its employees (Hasegawa et al., 1973). Nor did Hashimoto (1974) observe any abnormalities among 236 workers in such a facility, to whom he administered "examination centered around liver function tests."

In 1972, when Hasegawa et al., (1973) performed a health survey of workers in carbonless copy paper factories, i.e., 2 years after the use of PCBs in such processes had ceased, they noted no dermal effects, and no liver function, blood, or urine test abnormalities were seen, with the exception of "slight abnormalities in lipid metabolism". PCB levels in the blood of these workers were reported as "approximately 0.01-0.02 ppm," or what amounted to a decrease to 10% of the levels during the period of PCB usage. The authors' data indicated that PCB's collected from the work atmosphere had apparently degraded to a product containing one less chlorine atom than the average number of chlorine atoms in the PCB's handled in the factory, and that, conversely, the PCB's found in the workers' blood contained one more chlorine atom than did the PCB's handled in the factory, i.e., if the PCB's found in the workers' blood were inhaled at the workplace, it can be surmised that the di- and trichloro- biphenyls disappeared rapidly from the body, whereas the tetrachlorobiphenyl was metabolized slowly.

MONS 056401

Hasegawa et al., (1973) concluded that blood analysis is a feasible method for determining body burdens of PCBs. and "probably more useful than the technique presently used by extracting adipose tissue and using it for PCB;" urinalysis for "PCBs did not appeal to them as a viable monitoring method. They noted, however, that there was no correlation between length of employment (and presumably, duration of exposure) and the amount of PCBs accumulated in the blood, i.e., with continued exposure, PCB levels in the blood did not increase linearly; in light of the demonstrated slow excretion rate of PCBs from the body, the authors conjectured that some different biotransformative mechanism comes into play after blood PCB levels exceed approximately 1 ppm (perhaps fat storage). This would seem to belie their confidence in blood analysis as a reliable index of exposure.

Kitamura et al., (1973) reported that the mean PCB level in the blood of 10 capacitor factory workers was 0.82 ppm (0.32-2.1 ppm) immediately after the cessation of PCB usage, and 0.31 ppm 3 months later, when almost all of the subject were observed to have skin signs. It seems significant that the blood PCB concentrations observed in this study were several fold higher than those seen by Hasegawa et al., (1973). Kitamura et al., (1973) attributed the skin effects to PCB's accumulated in the workers' bodies during the period of PCB usage. No other abnormalities were reported.

MONS 056402

Sagami et al., (1973) reported the case of a housewife in whom they observed skin effects several years after she had left 6 years of employment in a capacitor factory. She was found to have PCB concentrations of up to 0.13 ppm in her blood and of 42 ppm in samples her subcutaneous adipose tissue; this contrasted sharply with the findings of Hara et al (1974) that the blood PCB levels of 3 workers who remained at the same factory had decreased to 0.05 ppm and below, one year after the cessation of PCB usage (from levels of 0.05-0.3 ppm during the time of PCB usage (Hasegawa et al., 1973).

N.B. Some capacitor manufacturers in the U.S. use epoxide-type alkylating agents as additives to prolong the service life of PCBs. EPA sec. 308 responses from the General Electric Co., probably contain identifying data.

VI. Air and Water Exposure to PCBs

As indicated previously, nominal human exposure to PCB's in the U.S. population may occur from air and water. Samples of ambient air were collected in suburban areas of Miami, Florida; Jackson, Mississippi; and Fort Collins, Colorado. Preliminary results (Kutz and Yang, 1975) for samples taken in April, May and June of 1975 show that PCBs were present at all locations. Although the data varied, the average concentrations at each of the three locations was approximately 100 nanograms per cubic meter. Initial

MONS 056403

identification of the PCBs indicated that they were most comparable to the Aroclor 1254 standard.

Dennis (1975) has reported that data gathered from monitoring activities of surface waters and bottom sediments of the major drainage basins of the United States indicate the widespread occurrence of PCBs in both surface water and bottom deposits. A preliminary assessment of PCB levels shows mean residue levels in water ranged from 0.01 to 0.05 mcg/liter. The 0.05 mcg/liter were found in the South Atlantic Slope and Eastern Gulf of Mexico drainage basin. In general, the lowest PCB residue levels were found in drainage basins west of the Mississippi.

Kleinert (1975) has summarized the work completed by the Wisconsin Department of Natural Resources to identify some PCB sources in the environment in Wisconsin. Effluents from cooling water in aluminum foundries contained PCBs ranging from 11.5 to 335 ppb. Investigations revealed the common source to be leaking hydraulic fluids containing PCBs which were used in die cast machines. Effluents from paper mills that recycle wastepapers has measureable discharges ranging from 0.01 to 25 ppb.

Snow samples (Kleinert, 1975) were collected early in 1975. Analysis of the snow melt water from Racine, Kenosha, Madison, and Milwaukee revealed concentrations from 0.17 to 0.24 ppb. The author concludes that these values suggest that fallout of PCBs from the air may be a principal source of PCBs entering the waters

MONS 056404

of the State.

Hesse (1975) indicates that similar to the studies in Wisconsin, not all industrial effluents tested in Michigan contain measureable PCB levels. In testing over 900 industrial samples, approximately 40 percent contained PCBs above the 0.1 mcg/liter laboratory sensitivity limit. Twenty-three percent of the industries tested had greater than 0.5 mcg/liter, 18 percent greater than 1 mcg/liter, 6 percent greater than 10 mcg/liter and 2 percent greater than 100 mcg/liter.

Although much of the PCBs entering municipal waste treatment facilities are removed and become incorporated into the waste sludge, a sampling of 58 municipal wastewater treatment plant effluents throughout Michigan in 1973 showed an average concentration of 0.52 mcg/liter. Concentrations of PCBs are much higher in the sludges. The average for all plants was 15.6 mg/kg with individual values as high as 350 mg/kg. Since sewage sludges are commonly disposed of by incineration, spreading on agricultural land, or placing in landfills, the addition of PCBs to the environment is obvious.

VII. Residues of PCB's in Human Tissue and Milk

Yobs (1972) is reported that 31.1 percent of 637 samples of human adipose tissue collected from the general population as part of the Human Monitoring Survey during 1971 were positive for PCBs in measureable amounts. These samples were collected in 18 states

MONS 056405

and the District of Columbia and positive samples were obtained from each of the sampling states and District. The percentage of PCB levels ranged from 34.2 percent non-detected, 33.3 percent less than 1 ppm, 27.3 percent 1-2 ppm and 5.2 percent greater than 5.2 ppm.

Kutz and Strassman (1975) have described the results of PCB monitoring during fiscal years 1973 and 1974 in which 35.1 percent and 40.3 percent, respectively, of the tissues collected contained levels of 1 ppm or more of PCBs on a net-weight basis. Analysis of the tissue revealed that the compounds found in adipose tissue were most comparable to those prevalent in Aroclor 1254 and Aroclor 1260. Additional analysis indicated that the most frequently encountered PCB residues were petan-, hexa-, and heptachlorobiphenyl compounds.

Residues of PCBs have also been detected in a study of human milk collected in Colorado where 8 or 40 samples contained residues ranging from 40 to 100 ppb (Savage et al., 1973).

A study of adipose tissue samples collected at autopsy from Canadians (Grant et al., 1975) indicates that the majority of Canadians have adipose tissue residues of 1 to 2 mg/kg of PCB. All adipose tissues had detectable levels of PCBs and 30 percent of the samples had PCB residues greater than 1 mg/kg with a range

MONS 056406

of 0.11 to 6.60 mg/kg. PCB residues in human milk from Ontario residents were found to be approximately 1 mg/kg on a fat basis.

MONS 056407

HUMAN EXPOSURE TO THE POLYBROMINATED BIPHENYLS

I. Introduction and Background

The polybrominated biphenyls (PBBs) in this report refers to either Firemaster BP-6 or hexabromobiphenyl manufactured by Michigan Chemical Corporation for use as a flame retardant for thermoplastics. This product is a mixture of brominated biphenyls with an average bromine content equivalent to about six bromine atoms per biphenyl molecule. Firemaster BP-6 is a mixture of the following brominated biphenyls[1]:

Tetrabromobiphenyl 2.0%

Pentabromobiphenyl 10.6%

Hexabromobiphenyl 62.8%

Heptabromobiphenyl 13.8%

Other bromobiphenyls 11.4%

The Michigan Chemical Corporation has stated that to their knowledge Firemaster BP-6 is the only polybrominated biphenyl produced in commercial quantity in the U.S. Their production estimates are:

Year	Pounds
1970	20,000
1971	200,000
1972	2,300,000
1973	3,900,000
1974 (Projected)	4,800,000

MONS 056408

Firemaster BP-6 has been used as a flame retardant in the manufacture of typewriter, calculator and microfilm reader housings, radio and TV parts, miscellaneous small automotive parts and small parts for electrical applications. The use of Firemaster BP-6 has been restricted to those applications where the end-use product is not exposed to either animal feed or food and there is no known use in flame retarding fabrics where human exposure would occur (1).

The ultimate disposition of Firemaster BP-6 upon burial is uncertain. The Michigan Chemical Corporation has stated that in their opinion this material will eventually undergo oxidative/biological degradation forming carbon dioxide, water and bromide ion (1).

II. Human Exposure

In October of 1973, adverse health effects were observed in cattle in several dairy herds in the State of Michigan. At that time, the cattle refused to eat manufactured feed; milk production decreased; there was a loss in body weight and the cattle developed abnormal hoof growth with lameness; cattle and swine aborted; and farmers reported the inability to breed heifers after they consumed feed manufactured by Farm Bureau Services. A herd of some 100 head of cattle sent to slaughter during this time period. exhibited enlarged livers.

MONS 056409

Analysis of samples of the suspected feed by laboratories of the U.S. Department of Agriculture at Beltsville, Maryland, revealed that the feed was contaminated with a flame retardant chemical, hexabrominated biphenyl. Subsequent investigation revealed that the Michigan Chemical Corporation manufactured magnesium oxide, a dairy feed supplement sold under the tradename Nutrimaster, and a flame retardant, hexabrominated biphenyl, sold under the tradename Firemaster BP-6. Both of these products were distributed in brown paper bags with either the name Nutrimaster or Firemaster stenciled across the top of the bag. When the top of the bag was torn off and discarded, identification was essentially lost.

As the result of a mix-up in bags, Firemaster BP-6 was mixed with animal feed in place of the Nutrimaster, apparently in the same proportion of use for the Nutrimaster. It appears that three kinds of feed were initially involved in this episode with PBB levels as follows:

Feed No.	PBB (ppm)
405	2.4
410	1,790
407	4,300

Samples of milk collected from individual farms soon after the PBB was identified as the contaminant ranged from 2.8 ppm on

MONS 056410

a fat basis to 270.5 ppm on a fat basis. Other products seized and destroyed included:

Butter	Range 1-2 ppm
Cheese	1.4 - 15.0 ppm
Canned milk	1.15 - 1.62 ppm

It has been estimated that between the onset of contamination in the fall of 1973 and the establishment of the quarantine of affected herds and flocks in the spring of 1974 over 10,000 Michigan residents have been exposed to PBB through the consumption of contaminated milk, meat and other dairy products. A considerable amount of variation in exposure has proabably occurred in both length of exposure and levels of exposure. As a group, the farm family members have been at greatest risk followed by those individuals who purchased dairy products from contaminated farms on a regular basis.

In order to determine whether or not persons exposed to PBB-contaminated products had suffered any acute adverse health effects, the Michigan Department of Public Health undertook a series of studies in the summer and fall of 1974. Study participants for the exposed group were dairy farm residents from farms which had been quarantined by the Michigan Department of Agriculture. The exposed subjects were limited to those who had lived or worked on the quarantined dairy farms for more than six months since May

MONS 056411

of 1973. Non-exposed subjects were randomly selected from a list of dairy producers in the same geographical area where farms had not been quarantined.

A total of 298 persons were interviewed in the study and physical examinations and/or blood samples were obtained for 110 persons in the exposed group and 104 persons from the control group.

The Michigan Department of Public Health has reported (1975) that responses to a set of 24 specific medical conditions revealed that none of the health complaints occurred consistently in either of the study groups. Statistical analysis showed that none of the listed complaints was significantly more frequent in those persons with the highest PBB levels when compared to other study subjects. Physical examinations of adults and children showed no unusual abnormalities of the heart, liver, spleen or nervous system. Urinalyses and complete blood counts did not reveal a significant excess of unusual abnormalities related to exposure or PBB levels.

These studies showed that blood levels of PBB were significantly higher in the study subjects from quarantined farms as compared to those from the non-quarantined farms; although some subjects from the farms showed low PBB blood levels (Table 18).

Several exposed females delivered normal babies without complication. Tests showed concentrations of PBB in breast milk to be considerably higher than that found in paired blood plasma (Table 19).

MONS 056412

Table 18

Distribution of PBB Blood Levels, Michigan, 1974

PBB Blood Levels (ppm)	Quarantined Farms Adults No.	%	Children No.	%	Nonquarantined Farms Adults No.	%	Children No.	%
0	3	3.7	-	-	21	28.4	-	-
0.002 - 0.019	43	52.4	8	28.6	52	70.3	29	96.7
0.020 - 0.090	19	23.2	10	35.7	1	1.4	1	3.3
0.100 - 0.490	11	13.4	3	10.7	0	0	0	0
0.500 - 2.260	6	7.3	7	25.0	0	0	0	0
TOTAL	82	100.0	28	100.0	74	100.1	30	100.0

Table 19

Comparison of PBB Concentrations In Human Breast Milk and Blood Plasma

Date	PBB Levels (ppm) Breast Milk	Blood Plasma
12/74	10.800	.082
6/74	22.700	.252
10/74	1.800	.014
3/75	.210	.003
3/75	92.660	1.068

Paired samples of adipose tissue and blood were collected in a group of 13 individuals entering the hospital for surgical procedures. The concentration of PBB in adipose tissue ranged from 61 to 370 times the PBB value found in blood plasma with an average ratio of 175 to 1 (Table 20).

MONS 056413

TABLE 20

Comparison of PBB Concentrations in
Human Adipose Tissue and Blood Plasma

Date	PBB Levels (ppm) Adipose Tissue	Blood Plasma
11/4	.410	.002
6/74 - 1/75	1.400	.005
3/75	3.000	.012
3/75	174.000	1.068
3/75	.248	.002
3/75	.274	.003
4/75	.177	.003
4/75	.152	.003
4/75	1.140	.004
4/75	.808	.004
4/75	.530	.007
4/75	.210	.003
5/75	1.110	.003

Reports of health complaints such as numbness, stomach pain, headache, fatigue and anxiety continue to be reported in the various newspapers in Michigan. Reports have also appeared in the press that several physicians in Michigan have reported abnormal liver function tests in patients exposed to PBB. Attempts to verify these reports with physicians have been unsuccessful.

MONS 056414

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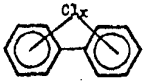
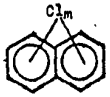
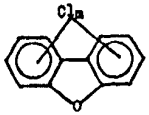
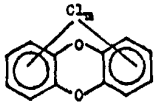
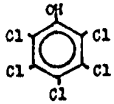
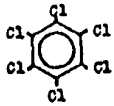
MONS 056443

Appendix A
CHLORINATED AROMATIC COMPOUNDS
REFERRED TO IN CHEMISTRY REPORT

NAME	STRUCTURE	EXTENT OF CHLORINATION POSSIBLE	NUMBER OF CHLORINATED DERIVATIVES POSSIBLE
Chlorinated biphenyls		x= 1-10	209
Chlorinated naphthalenes		m=1-8	75
Chlorinated dibenzofurans		m=1-8	135
Chlorinated dibenzo-p-dioxins		m=1-8	75
pentachlorophenol		-	-
hexachlorobenzene		-	-

MONS 056444

Calculation of dosages received by monkeys in reference 16

The amount of food eaten in relation to body weight varies between different animal species, including man. To enable comparisons, food or food component intake frequently is standardized by expressing the amount eaten as a function of body weight. The latter is known as the dosage, while the amount eaten would be the dose.

Table 1 lists mean body weights for all experimental animals. These range from 5.57 kg pretest to 4.84 kg at the end of 5 months on test. An overall mean body weight of 5.32 kg. was estimated by averaging the six figures listed.

Table 3 shows total 6-month polychlorinated biphenyl intake as 85.29 mg for 2.5 ppm group and 175.36 mg for the 5.0 ppm group.

Average daily intake was calculated by use of the following formula:

$$\mu g/kg/day = \frac{\text{total mg intake}}{\text{body wt. (kg)}} \times \frac{1}{180 \text{ days}} \times \frac{1000\ \mu g}{mg}$$

At 2.5 ppm, the dosage was approximately 89 µg/kg/day

At 5.0 ppm, the dosage was approximately 183 µg/kg/day

For comparison, FDA estimated safe intake as 1 µg/kg/day (14)

Thus, even though the monkey dietary levels of 5.0 ppm and 2.5 ppm were described as levels "equal to, and 50% of, the concentrations allowed in certain foods destined for human consumption" (16), it appears that the monkeys actually received about one hundred and two hundred times the dosages which FDA estimated as safe from the human experience at Yusho.

EVALUATION OF CHANGES OF THE LEVEL OF POLYCHLORINATED BIPHENYLS (PCB) IN HUMAN TISSUE

Final Report on FDA Contract 223-73-2209

Michigan Department of Public Health
Lansing, Michigan

Harold E. B. Humphrey, Ph.D.
Project Director
1-517-373-2037

Attachment 2-7

MONS 056446

EVALUATION OF CHANGES OF THE LEVEL OF POLYCHLORINATED BIPHENYL IN HUMAN TISSUE

SUMMARY OF FINDINGS

A two year study was made of persons who regularly consumed Lake Michigan sport fish and randomly selected persons who did not consume such fish. A total of 161 adults from the shoreline communities of Traverse City, Manistee, Ludington, and South Haven participated. 105 of these participants consumed more fish than recommended by the Michigan Department of Public Health (no more than one meal per week or 24 pounds per year) and constituted the PCB "exposed" group. The 37 participants eating six pounds or less per year, 19 eating an intermediate amount, and 19 fish eaters from Algonac where sport caught fish are low in PCB were used for comparison groups. A short medical history, a dietary record, and blood specimens were obtained for all participants. The following conclusions were reached from these data:

1. PCB blood levels ranged from 0.007 ppm in persons who ate no fish to a maximum of 0.366 ppm for a person eating Lake Michigan fish.

2. There was a direct relationship between the size and quantity of sport species of fish eaten and the PCB levels found in human blood.

3. In 1973, those who annually consumed 24 or more pounds of sport fish from Lake Michigan had a mean blood PCB value of 0.073 ppm; persons annually eating 6 pounds or less had a mean level of 0.020 ppm; persons who ate no fish averaged 0.017 ppm; and persons eating 24 or more pounds annually from Lake St. Clair had an average blood level of 0.023 ppm. The PCB blood levels observed for the Lake Michigan study groups were similar in 1974.

4. The level of PCB found in the blood of participants did not change significantly from year to year, nor did it diminish significantly when fish consumption was eliminated for up to nine months.

5. The PCB levels observed in cooked fish were lower than expected from reported levels in raw fish.

6. Eighty-two percent of those who exceeded the state's recommended maximum intake (no more than one meal per week or 24 pounds per year) of Lake Michigan fish received an estimated annual dose of PCB which was greater than that cited by the Food and Drug Administration as the maximum annual dose of PCB which should be received over a protracted period of time. This estimated intake ranged from 0.49 µg PCB/Kg body weight/day to 3.94 µg PCB/Kg body weight/day and averaged 1.7 µg/Kg body weight/day. The Food and Drug Administration recommends that PCB intake not exceed 1 µg/Kg body weight/day for long term exposure.

7. As a group, the exposed participants had no health problems or medical conditions that could be correlated with PCB blood levels, exposure to Lake Michigan fish, or known symptoms of PCB poisoning.

CONCLUSION:

Consumption of Lake Michigan fish contributed substantially to the PCB levels in the blood of humans. The dose received is insufficient to produce a detectable effect on human health at present. It has not been determined whether long term exposure to PCB contaminated fish will result in a continuing accumulation of PCB's or eventually produce identifiable health effects in humans. The data gathered to date appear to justify continued surveillance of this situation in Michigan. The data also justify a continued recommendation that intake of Lake Michigan salmon and lake trout be limited to less than one meal per week or 24 pounds per year.

TABLE OF CONTENTS

MONS 056449

LIST OF TABLES

MONS 056450

LIST OF TABLES (continued)

MONS 056451

LIST OF FIGURES

MONS 056452

LIST OF FIGURES (continued)

MONS 056453

EVALUATION OF CHANGES OF THE LEVEL OF POLYCHLORINATED BIPHENYL IN HUMAN TISSUE

INTRODUCTION

Polychlorinated biphenyls (PCB) have been manufactured and used for over forty years in a wide variety of industrial applications which require a substance with chemical and thermal stability. Approximately 40% of these uses may have resulted in loss of PCB to the environment. PCB residues began to be identified in animal and environmental samples in the late 1960's and it was soon confirmed that this chemical was a persistent and a ubiquitous world wide environmental contaminant (1, 2). PCB's have been detected in manufactured products, air, water, snow and sediment samples as well as aquatic biota and terrestrial animals and birds. A consequence of this situation has been concern about the indirect contamination of some foods destined for human consumption and the resulting exposure of humans to PCB. Reports of the detection of low levels of PCB in tissues from the general population (3, 4) have confirmed that human exposure is a reality.

The human health effects from PCB exposure, especially long term low level exposure, are not clearly understood. Information on PCB exposure for humans has been chiefly confined to acute effects from industrial exposure and an extensive food contamination episode in Japan where a disease called Yusho has been described (7). Although reports on the toxic symptoms observed with Yusho have generally been accepted as attributable to PCB poisoning, the suspected existence of other toxic contaminants in Japanese PCB preparations has raised questions on the cause of some of these observed effects.

MONS 056454

Scientific information on the characteristics and toxicity of PCB's was reviewed in 1972 (2), and currently by the Food and Drug Administration (FDA) with respect to the adequacy of the temporary tolerances for PCB (9). The body of data reported to date indicates that the toxicity of PCB varies with the extent of chlorination of the biphenyl molecule. These compounds are poorly metabolized and tend to accumulate and be retained in the lipid portion of animal tissues for prolonged periods (5, 10, 11). The accumulation and storage of a chemical would theoretically make it available, under appropriate conditions, for recirculation within the body allowing continuous or repeated exposure of target organs long after the original dose has ceased or when multiple low doses are received and accumulated to a critical level. The significance of the observed accumulation and storage of PCB in tissue is not fully known. However, recent reports of the continued presence of some clinical signs in Yusho patients years after the incident (5, 12) and the detection of significant levels of PCB in rat tissues ten months after exposure (11) tend to support the storage - continued exposure contention. Moreover, recent studies reporting acute health effects including reproductive failure in primates fed low levels of PCB (13, 14), the induction of liver tumors in rats from PCB ingestion over prolonged periods (15), and the partial conversion of tetrachlorobiphenyl to a more acutely toxic monohydroxy-metabolite in rats (16) suggests that long term or low level exposure to PCB or its metabolic byproducts is not fully understood and may be of significance.

The existing body of toxicity data for PCB has been sufficient for the FDA to regard these compounds as potentially serious health hazards in food. Temporary tolerances for PCB's were established by the FDA in 1973 (8). The determination of tolerance levels was predicated on the assumption that PCB exposure from food was sporadic and would diminish with time. Subsequent surveillance data has confirmed this to be true

MONS 056455

except for fresh water fish found in certain areas of the nation (9). It has been concluded that frequent consumption of fish containing PCB in excess of 5 parts per million (ppm) poses a risk to public health. Data for the evaluation of this risk to humans has not been available. Concern about the risk and the need to obtain information on humans exposed to PCB's from consumption of contaminated fish was the basis for establishing the research contract reported below.

THE PCB PROBLEM IN MICHIGAN

The Great Lakes surrounding Michigan represent one of the areas of the nation contaminated with PCB. The Michigan Water Resources Commission has reported that many surface water samples contained PCB at concentrations above the detection limit of 10 parts per trillion (ppt). Significant levels of PCB have been found in rivers and streams discharging into the Great Lakes. Effluents from wastewater treatment plants servicing industrialized communities have been found to be highly contaminated. Although efforts have been made to eliminate point source industrial losses of PCB, recent monitoring reveals that PCB contamination continues to be a problem. It has been suggested that highly contaminated bottom sediments in sewers and receiving streams may represent a reservoir for the continued release of PCB. It has been concluded that PCB continues to be discharged into the Great Lakes from many small but diffuse sources including atmospheric fallout.

PCB's were first detected in Great Lakes fish in 1969 when substances interfering with the analysis of DDT in Coho salmon samples were determined to be PCB (3). Subsequent environmental monitoring has identified

MONS 056456

PCB contamination in fish from all of the Great Lakes. The problem is most severe in Lake Michigan and least severe in lakes Huron (except for Saginaw Bay) and Lake St. Clair. Concern for PCB contamination has focused on Lake Michigan because of the large investment in the rejuvenation of this lake for sport fishing through fish stocking programs.

Statistical sampling surveys of contaminants in Great Lakes fish began in 1972. This program has shown that most species tested contained detectable levels of PCB's and that contamination levels were generally proportional to fish size (age) and highest in the predator species. Except for whitefish, the species of commercial or sport interest (trouts and salmons) from Lake Michigan were found to be most highly contaminated with PCB. Moderate and larger sized specimens have been found to be well in excess of the FDA tolerance level of 5 ppm. A portion of the data obtained for fillets of lake trout from Lake Michigan during the 1974 Great Lakes Environmental Contaminants Survey is exemplified below:

Lake Zone	(Size) Pounds	No. Fish	Mean PCB (ppm)
MM4	5-10	3	3.06
MM5	5-10	7	9.01
MM6	5-10	20	9.08
MM7	5-10	11	11.86
MM8	5-10	6	11.93

The lake zones refer to the statistical sampling areas of Lake Michigan from which the specimens were taken and are identified in Figure 1. The values shown indicate a trend of increasing PCB levels as samples are obtained further south in the lake along more heavily populated and industrialized areas of the State.

MONS 056457

Unfortunately PCB contamination of fish in the Great Lakes has not decreased to date. Table 1 shows data from a three-year study of two species of Lake Michigan fish by the U.S. Fish and Wildlife Service Laboratory. As shown, the PCB levels in lake trout appear to have risen and have not declined in Coho salmon over the study period.

TABLE 1. PCB CONTAMINATION IN WHOLE FISH*

Species & Location	Collection Year	Length (inches)	Sample Size	Mean PCB Value (ppm)
Lake Trout near South Haven	1972	20 - 28	9	12.86 ± 4.75
	1973	20 - 28	30	18.93 ± 2.08
	1974	20 - 28	30	22.91 ± 3.75
Coho Salmon near Ludington	1972	20 - 32	10	10.93 ± 2.12
	1973	20 - 32	29	12.17 ± 0.77
	1974	20 - 32	30	10.45 ± 0.92

*Data from the Great Lakes Fishery Laboratory, U.S. Bureau of Sport Fisheries and Wildlife.

The continued discharge of PCB into the water and trend analyses such as these indicate that PCB contamination of Great Lakes fish is a long term problem.

Sport fishing represents an uncontrolled source of PCB contaminated fish for distribution and human consumption. The State and federal inspection system controls exposure to PCB from commercial sources by seizure of contaminated fish before it reaches the market. Sport fishermen and those who consume sport caught Great Lakes trout and salmon represent the population most likely to be at risk to significant PCB exposure from fish. The Michigan Department

MONS 056458

TABLE 2. 1974 CATCH AND EFFORT REPORT FOR LAKE MICHIGAN*

	SPECIES					
	Lake Trout	Rainbow - Steelhead	Brown Trout	Coho Salmon	Chinook Salmon	TOTAL
No. Caught/ Year	739,260	271,980	96,480	502,200	264,420	1,874,320
Mean Weight/ Fish	5-6	8-10	5	6	15	-
Pounds of Fish Caught/ Year	4,065,930	2,447,820	482,400	3,013,200	3,966,300	13,975,650
No. Pounds/ Fisherman/ Year	10.6	6.4	1.3	7.9	10.4	36.6

* Data from the Michigan Department of Natural Resources 1974 Annual Sport Fishing Survey. Information obtained from statistical quarterly census of the 381,600 licensed fishermen in the 18 Michigan counties bordering Lake Michigan.

MONS 056459

Natural Resources indicates that 381,000 licensed fishermen reside in the 18 counties bordering Lake Michigan. That department's 1974 sport fishing survey (Table 2) shows that this group catches about 14 million pounds of trout and salmon from Lake Michigan annually. As indicated, the average size of the fish being caught fall into the range which is significantly contaminated with PCB. Statistically, each of these fishermen obtains 36 pounds per year (3/4 pounds per week) of Lake Michigan fish for consumption. This is over three times the reported national per capita consumption of fish from commercial sources (11.8 pounds) but about half that reported for the Japanese (67.6 pounds) (Source: Production Yearbook, 1970, vol. 24, Food and Agriculture Organization of the United Nations). Although the habits of individuals will vary it appears that as a group these sport fishermen consume more fish than the average and are successfully catching the PCB contaminated species from Lake Michigan.

THE MICHIGAN PCB STUDY

The Food and Drug Administration Contract 223-73-2209 with the Michigan Department of Public Health represents an attempt to evaluate human exposure to PCB from consumption of sport caught Lake Michigan fish. The study was principally designed to provide information on the levels and changes in the level of PCB's in humans. It was not intended to be a full toxicological evaluation of the human health effects of PCB exposure. The major objectives of the study were:

1. To conduct research on the analysis of PCB's in order to develop a sensitive laboratory method for detecting PCB concentrations in human blood.

2. To identify exposed and control population groups in selected geographical locations where PCB levels in fish are excessive.

MONS 056460

3. To select participants for the study and collect suitable blood specimens using accepted epidemiologic techniques.

4. To evaluate the concentration and variation of PCB's in representative groups of humans.

5. To estimate the fish consumption characteristics of the selected exposed group.

6. To determine the PCB levels in fish being consumed by humans.

7. To determine by laboratory analysis the concentration of PCB's in participants' blood specimens in order to evaluate:

   A. The PCB ingestion from fish

   B. The PCB levels in the blood of humans who eat fish and who do not eat fish

   C. The variation of PCB levels in blood due to seasonal fish consumption habits

   D. The metabolic half-life of PCB's present in the blood of humans

8. To attempt to correlate levels of PCB in tissues with levels of PCB ingestion.

FIELD SURVEY DESIGN:

The greatest opportunity for exposure to PCB's, as discussed above, was believed to lie in the consumption of certain sport caught species of Lake Michigan fish. Persons from four Michigan communities, South Haven, Ludington, Manistee, and Traverse City, all on the shoreline of Lake Michigan (see Figure 1) were selected for the study. These communities are points of origin for Great Lakes sport fishing and span the principal areas of Lake Michigan where PCB contaminated

MONS 056461

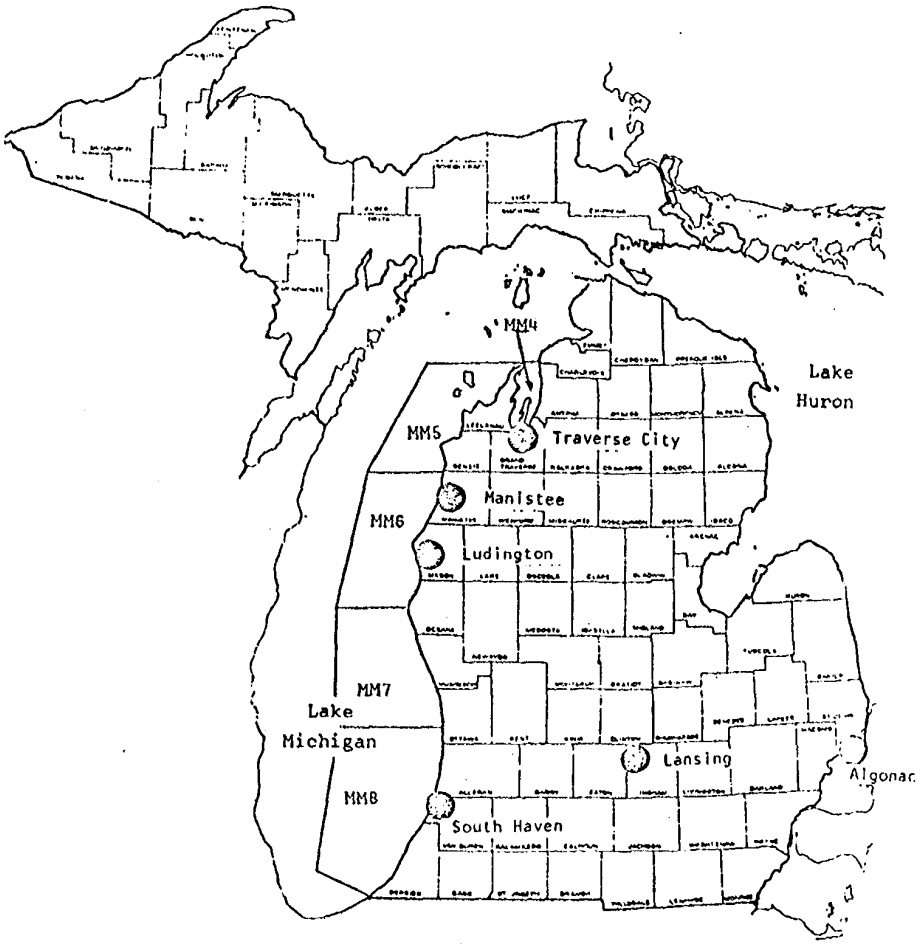


FIGURE 1. LOCATION OF PCB EXPOSED & CONTROL PARTICIPANTS

MONS 056462

fish have been identified. In addition, Algonac, a shoreline community on Lake St. Clair where PCB levels in fish are not excessive was included for a comparison of fishermen from a different geographical location.

Exposed and control subjects for the study were personally selected by field workers following introductory publicity in the local news media of each community. Adults (age 18 and above) who caught and regularly ate sport fish from Lake Michigan were sought and encouraged to participate. Exposed participants, those consuming at least 24-26 pounds of Great Lakes fish per year (an annual average of at least one 8 oz. meal per week) were identified through a voluntary and referral system. Control participants, those consuming less than six pounds of Great Lakes fish per year (an annual average of zero to no more than one 8 oz. meal per month) were identified by contacting households randomly selected from the directory for each community. All potential participants, whether voluntary or randomly selected, were visited and personally interviewed in order to determine their eligibility for the study utilizing the above fish consumption criteria and a questionnaire developed for the study. Those judged eligible by the interviewer, as determined by the responses to the fish consumption questions, were invited to participate in the study. Persons in agreement were then briefed on the purpose and extent of the study, were fully interviewed, given a bound log for recording their subsequent consumption of Great Lakes fish, and were asked to freeze portions of their fish meals. In each case the interviewer explained the importance and method for these procedures.

Periodic contacts were made in order to maintain participant interest in the project and to schedule blood sample collections. Specimen collections for laboratory analysis were scheduled at the local health department offices at times convenient for the participants. Whole blood samples (20 cc) were collected by venipuncture by the

MDNS 056463

Michigan Department of Public Health staff. During these appointments, questionnaire information was updated and an informed consent statement was signed by the participant.

Exposed and control populations in South Haven, Ludington, Manistee and the Traverse City area were identified and selected for the study as described above. Blood specimens were collected from 152 persons during the Fall of 1973 at the end of the fishing season on the Great Lakes. This sampling provided a baseline for evaluation of PCB values after consuming sport fish through a whole fishing season. The classification and location of these participants are shown in Table 3. The representative number of randomly selected unexposed controls to be sought from each community was determined by consultation with statisticians. The group classified as "intermediate" represents persons who qualified as fish eaters when initially interviewed, but failed to actually consume a sufficient quantity of Great Lakes fish during the 1973 season or the 1974-75 season to be classified as exposed fish eaters.

Blood specimens were collected during the Spring of 1974 (April sample, Table 4) from seventeen of the Traverse City area fish eaters who had been sampled the previous Fall. These collections were made prior to the resumption of sport fishing for the 1974 season and represent an attempt to evaluate the seasonal variation in the level of PCB's in exposed persons. Consumption of frozen or canned Great Lakes fish during the winter months represents a problem for the evaluation of seasonal variation. It was originally believed that consumption of sport fish would cease shortly after the fresh supply was exhausted at the end of the fishing season. However, work with the study participants revealed that fresh caught sport fish are preserved, frozen or canned, to a much greater extent than expected. Thus, the cessation of fish consumption is not

MONS 056464

TABLE 3. PCB STUDY, PARTICIPATION BY CITY

CITY	Number Participants 1973				Number Participants 1974-75				Total Number Participants Over Life of Study			
	Total	Control	Inter-mediate	Exposed	Total	Control	Inter-mediate	Exposed	# Indi-viduals	Sex M	Sex F	Media Age
Traverse City	53	19	5	29	60	13	10	37	74	32	42	49.9
Manistee	21	7	2	12	21	5	3	13	25	13	12	45.9
Ludington	28	10	8	10	20	7	5	8	33	15	18	41.0
South Haven	31	2	4	25					31	21	10	42.9
Algonac	19	0	5	14*					19	13	6	51.1
TOTALS	152	38	24	90	101	25	18	58	182	94	88	45.1

*Exposure here constitutes consumption of fish from Lake St. Clair where PCB levels are relatively low.

MONS 056465

abrupt but instead tapers off slowly as the preserved supply is exhausted or continues at a reduced level until the following fishing season.

The 101 participants for the second (1974-75) phase of the study are listed in Table 3 by location. The July 1974 specimen collection (Tables 4, 5, 6) provided a second baseline sample for the study. A portion of these participants are persons who were included in the Fall screening from the previous year (1973). This provided for a year to year comparison of PCB levels in certain individuals. The balance of the 58 fish eaters were recruited during the Spring of 1974. Emphasis was placed on recruiting persons with a history and expectation of catching and consuming an amount of fresh fish well in excess of the required 24-26 pounds per year. This effort was in response to the realization that the quantity of fish consumption among Great Lakes fishermen is not consistent from year to year. Fish consumption appears to be dependent on the weather (number of trips onto the lake) and on success once at the fishing grounds. One of the problems encountered during this study was the inability of some exposed participants to catch and consume the anticipated quantity of fresh fish throughout the study. In some cases this necessitated a downward reclassification of the participant.

The 1974-75 phase of the PCB Project provided a second year comparison, a close monitoring of individual fish consumption over an entire fishing season, and an evaluation of the change in level of PCB's in blood through a series of specimen collections after the voluntary termination of fish consumption at the end of the season. Completion of the abstinence study was complicated by the staggered dates when individuals concluded one fishing season and began the next. In addition, the request for participants to cease eating sport fish represented a

MONS 056466

break in their normal habit of consuming frozen fish through the winter. These problems resulted in the elimination of some participants from this portion of the study, because they failed to abstain from eating fish for a sufficient period of time.

STUDY FINDINGS:

The study was designed to include 140 to 160 participants. This goal was achieved as 182 persons participated during the 2 years of the study. Table 3 shows the classification of participants in each community for the 1973 and 1974-75 phases of the study. The size of the control group was based on the number needed to be statistically acceptable for data evaluation. The intermediate exposure classification was necessitated by the realization that a difference existed between estimated fish consumption based on recall responses to the questionnaire and actual consumption of fish during the study period. As explained previously some persons who were alleged to consume over 24-26 pounds of Lake Michigan fish annually failed to do so and were reclassified from the exposed to an intermediate (7 to 23 pounds per year) group.

Many of the first year participants continued in the study to its conclusion. Those who elected to drop out and those who were reclassified as intermediate exposure after the first year were replaced by additional exposed participants for the second year of the study. Table 3 also shows that the distribution of males and females was nearly equal and that the median participant age was about 46 years. The age, sex, height, and weight data for each participant is provided in Appendix C.

MONS 056467

The quantity of fish consumed by individuals in the exposed classification was as a group unimpressive but correlated with the calculated mean catch as reported in the sport fishing survey (Table 2). The reported popularity and catch success of sport fishing on Lake Michigan and the deliberate effort to enlist reputed fishing enthusiasts in the study led to expectations that the exposed group would consume large quantities of fish. However, a plot of the distribution of mean annual fish consumption over the two fishing seasons of the study shows that the most frequently recorded quantity of fish actually consumed was in the 24-35 pounds per year range (Figure 2). The number of participants reporting a consumption of greater than 53 pounds was low. The highest reported consumption over the two year period was 180 pounds per year and the highest single season consumption was 260 pounds per year (data for annual consumption from Tables 4, 5, 6 & 7). The annual consumption for each fisherman based on a two year average was lower than that based on a single season because of the aforementioned inconsistency in fishing success from year to year. Although the reported size and species of fish being consumed indicates a significantly contaminated raw food source is being obtained, the average Michigan fisherman apparently does not consume high quantities of fish. The study did confirm that these people eat more fish than the reported national per capita average of 11 pounds per year and that some individuals consume significant amounts of contaminated fish.

PCB's were found in 500 blood and one breast milk specimens collected from participants during the study. The values ranged from a low of 0.007 ppm in the control group to a high of 0.366 ppm in the exposed group. A baseline sample for 1973 (November) and for 1974 (July), a spring sample in 1974, and a series of samples commencing in 1974 and ending in 1975 represent the planned collection schedule for the study. The annual fish consumption and the concentrations of PCB detected in blood collected from participants at the specified times during the study are shown in Tables 4 (Traverse City),

MDNS 056468

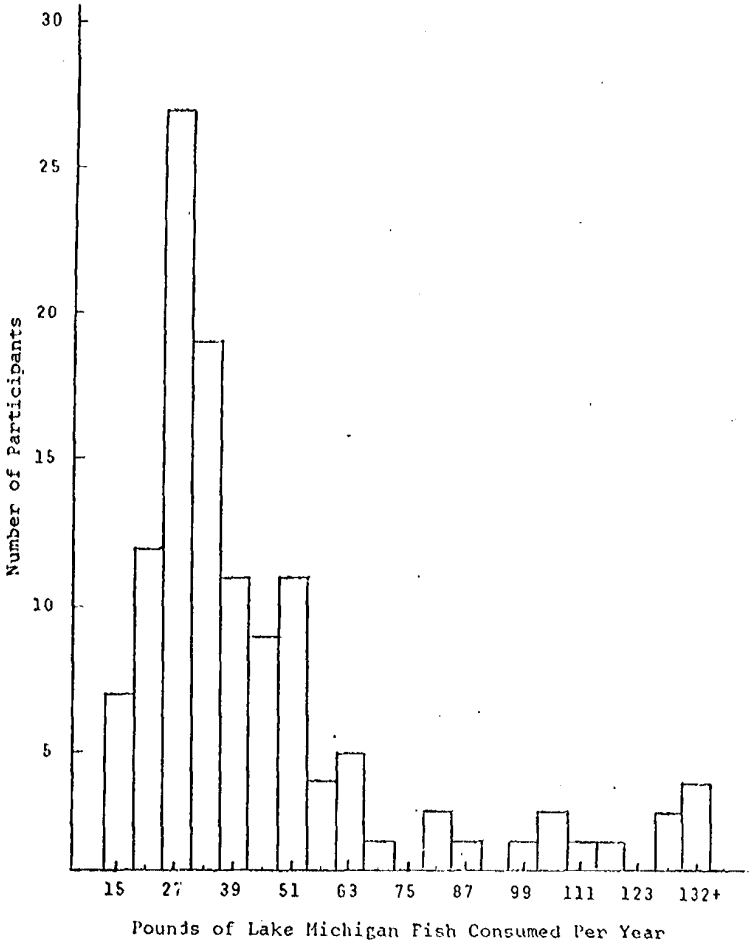


FIGURE 2. FREQUENCY DISTRIBUTION OF REPORTED GREAT LAKES FISH CONSUMPTION, EXPOSED GROUP, TRAVERSE CITY, MANISTEE & LUDINGTON

MONS 056469

5 (Manistee), 6 (Ludington), and 7 (South Haven and Algonac). The participants are listed in these tables by ascending order of annual fish consumption as reported for the 1974 baseline year. The raw laboratory data for all PCB tests run are recorded in Appendices A and B. The 35 test results shown in Appendix B are for specimens which were depleted during the development of the final analytical test procedure. These results have been segregated and are not presented in Tables 4, 5, 6 & 7 because it was not possible to retest these specimens with the more precise procedure which was adopted for use in the study.

There was a highly significant correlation between the reported quantity of Lake Michigan fish consumed and the concentration of PCB in the blood of participants in the study. Examination of the blood level and fish consumption data for persons from four communities bordering Lake Michigan (Tables 4, 5, 6 & 7) revealed 117 participants who reported eating fish from the lake in 1973. A plot of the levels of PCB in blood vs pounds of fish consumed for this group (Figure 3) indicates a wide range of blood values for each quantity consumed. Using a natural log transformation* of PCB values the data was again plotted (Figure 4) and the correlation between these variables determined to be 0.51 which is highly significant ($t = 6.24$, $p<<.001$). Thus higher reported fish consumption was associated with more PCB. In addition, a regression analysis of other variables (from Appendix C) showed that being older and male (height and weight correlated significantly with PCB and with being male) was significantly associated with higher PCB levels ($p<<.001$).

The direct relationship between the concentration of PCB in blood and increasing quantities of Lake Michigan fish consumed was observed in all four of the study communities

*A log transformation is used when variance increases as values increase (18).

Statistical computations were provided by the Office of Vital and Health Statistics, Michigan Department of Public Health.

MONS 056470

TABLE 4. CORRELATION OF FISH EXPOSURE WITH PCB VALUES IN BLOOD OVER A TWO YEAR PERIOD, TRAVERSE CITY GROUP.

I.D.#	1973 Fish Consumed	1974 Fish Consumed	BLOOD SPECIMENS - Total PCB in parts per million						
			Nov.'73	Apr.'74	Jul.'74	Jan.'75	Feb.'75	Apr.'75	May '75
0354	0 lbs.	0 lbs.	0.033		0.027				
0345*	0 lbs.		0.019						
0340*	0 lbs.		0.041						
0341*	0 lbs.		0.027						
0314*	0 lbs.		0.011						
0316	0 lbs.	0 lbs.	0.016		0.011				
0318	0 lbs.	0 lbs.	0.010		0.012				
0319	0 lbs.	0 lbs.	0.013		0.015				
0320	0 lbs.	0 lbs.	0.014		0.015				
0368	0 lbs.	0 lbs.			0.023				
0321*	1 lbs.		0.032						
0344*	1 lbs.		0.015						
0342	1 lbs.	1 lbs.	0.018		0.022				
0343	0 lbs.	1 lbs.	0.014		0.019				
0329*	2 lbs.		0.022						
0315	2 lbs.	2 lbs.	0.039		0.041				
0346	0 lbs.	2 lbs.	0.017		0.019				
0347	2 lbs.	2 lbs.	0.015		0.022				
0317	4 lbs.	3 lbs.	0.024		0.029				
0313	4 lbs.	5 lbs.	0.007		0.010				
0333	28 lbs.	8 lbs.	**	**	**	0.038		0.032	0.031
0332	32 lbs.	8 lbs.	**	**	**	0.051		0.040	0.061
0366	29 lbs.	9 lbs.		0.018	0.023	0.021	0.016	0.019	
0371		12 lbs.			0.025	0.036	0.025	0.026	0.037
0335	45 lbs.	16 lbs.	0.043		0.041		0.056	0.066	0.077
0360	18 lbs.	16 lbs.		0.019	0.023				
0348*	17 lbs.		0.045						
0349*	17 lbs.		0.020						
0327*	18 lbs.		0.022						
0355	37 lbs.	18 lbs.		0.041	0.043	0.052	0.044	0.043	
0308*	22 lbs.								
0334	40 lbs.	22 lbs.	0.040		0.053		0.051		0.052
0310	17 lbs.	22 lbs.	0.020		0.023	0.026			0.021
0324	35 lbs.	22 lbs.	0.041		0.058	0.053			0.039
0337	29 lbs.	24 lbs.	0.028		0.041		0.040	0.042	0.039
0306*	25 lbs.		0.040						
0363	27 lbs.	25 lbs.		0.036	0.061		0.039	0.032	0.040
0353	27 lbs.	30 lbs.	0.048		0.063	0.074	0.070	0.054	0.066
0352		30 lbs.	0.093		0.103	0.090	0.097	0.086	0.130
0365	40 lbs.	30 lbs.		0.014	0.015	0.012	0.012	0.013	0.013
0325	32 lbs.	31 lbs.	0.075		0.065	0.058	0.052	0.074	0.065
0301	57 lbs.	32 lbs.	0.038	0.028	***	0.033	0.026		
0369	33 lbs.	33 lbs.			0.036	0.036	0.034		
0305*	34 lbs.		***	**	**	0.013	0.019	0.013	0.029
0372	67 lbs.	34 lbs.	0.055		0.080	0.077			
0370		36 lbs.			0.034	0.036	0.041	0.056	0.032
0359	56 lbs.	39 lbs.		0.033	0.033				
0309	33 lbs.	40 lbs.	0.038		0.058	0.058			0.069
0302	51 lbs.	41 lbs.	0.085	**	**	0.056	0.050	0.058	0.074
0346	29 lbs.	41 lbs.	0.037		0.037		0.058	0.029	0.034

CONTINUED . . .

MONS 056471

(TABLE 4 - Continued)

I.D.#	1973 Fish Consumed	1974 Fish Consumed	BLOOD SPECIMENS - Total PCB in parts per million						
			Nov.'73	Apr.'74	Jul.'74	Jan.'75	Feb.'75	Apr.'75	May '75
0326	32 lbs.	44 lbs.	0.044		0.059	0.069	0.057	0.049	0.074
0362	48 lbs.	44 lbs.		0.034	0.034	0.031	0.036	0.027	0.032
0372		45 lbs.			0.045				
0312	48 lbs.	47 lbs.	0.065		0.064	0.062	0.047	0.045	0.061
0300*	51 lbs.		0.060		**				
0303*	51 lbs.		**	**	**	0.043			
0304*	51 lbs.		0.057						
0361	53 lbs.	53 lbs.		0.043	0.065	0.040	0.042		
0328	62 lbs.	53 lbs.	0.047	0.037	0.048	0.058	0.056	0.037	0.042
0330	68 lbs.	56 lbs.	0.030	0.044	0.042	0.040	0.045	0.036	0.045
0351	24 lbs.	60 lbs.	0.081		**	0.080			0.092
0323	67 lbs.	68 lbs.	0.050		0.058	0.078			0.071
0358		79 lbs.		0.142	0.131	0.177	0.152	0.135	0.147
0331	162 lbs.	90 lbs.	0.152	0.115	0.134	0.101	0.140	0.121	0.172
0357	260 lbs.	98 lbs.		0.022	0.026	0.021			0.028
0311	130 lbs.	111 lbs.	0.096	0.083	0.068				
0356	75 lbs.	120 lbs.		0.071	0.064	0.067	0.073	0.059	0.068
0350	93 lbs.	120 lbs.	**		**	0.106			0.121
0373	90 lbs.	131 lbs.			0.064	0.076	0.059	0.064	0.068
0367	162 lbs.	140 lbs.			0.093	0.123			0.086
0364	60 lbs.	147 lbs.		0.007	0.011				0.014
0339	85 lbs.	152 lbs.	0.083	***	0.072	0.086	0.088		0.082
0338	156 lbs.	162 lbs.	0.114		0.120	0.078	0.081	0.095	0.088

* Chronological order of fish consumed is based on 1973 data. The 1974 questionnaire was not completed.

** Sample was depleted during initial analysis, using decachlorobiphenyl techniques. See Appendix (B) for decachlorobiphenyl blood values.

*** Sample lost (broken, not received, etc.)

MONS 056472

TABLE 5. CORRELATION OF FISH EXPOSURE WITH PCB VALUES IN BLOOD OVER A TWO YEAR PERIOD, LUDINGTON GROUP.

I.D.#	1973 Fish Consumed	1974 Fish Consumed	BLOOD SPECIMENS - Total PCB in parts per million Nov.'73	Jul.'74	Dec.'74	Jan.'75	Mar.'75	Apr.'75	May '7
00203	0 lbs.	0 lbs.	0.011	0.014					
00204*	0 lbs.		0.017						
00206	1 lbs.	0 lbs.	0.021	0.021					
00207*	1 lbs.		0.018						
00200	3 lbs.	1 lbs.	0.037	0.042					
00201	3 lbs.	1 lbs.	0.029	0.031					
00209	2 lbs.	1 lbs.	0.016	0.016					
00202	3 lbs.	2 lbs.	0.015	0.018					
00205	2 lbs.	4 lbs.	0.020	0.029					
00208*	6 lbs.		0.030						
00219*	8 lbs.		0.046						
00220*	8 lbs.		0.016						
00221*	8 lbs.		0.023						
00212*	9 lbs.		0.026						
00218	22 lbs.	10 lbs.	0.023	0.022	0.026	0.029	0.026		0.023
00214	31 lbs.	12 lbs.	0.089	0.066	0.069				
00225*	14 lbs.		0.054						
00215	26 lbs.	15 lbs.	0.062	0.078	0.063	0.059	0.064		0.055
00227*	18 lbs.		0.079						
00230	18 lbs.	18 lbs.		0.055					
00217	29 lbs.	19 lbs.	0.060	0.071	0.048	0.047	0.052		0.064
00224*	19 lbs.		0.118						
00222*	26 lbs.		0.063						
00223*	26 lbs.		0.036						
00229	29 lbs.	26 lbs.		0.021	0.019	0.021	0.017	0.021	0.023
00216*	27 lbs.		0.031						
00228	27 lbs.	27 lbs.		0.031	0.033	0.022	0.017	0.021	***
00231	28 lbs.	28 lbs.		0.031	0.026	0.025	0.053	0.046	0.025
00210	24 lbs.	33 lbs.	0.065	0.076	0.085	0.070	0.070	0.084	0.085
00211	24 lbs.	39 lbs.	0.065	0.077	***	0.066	0.066	0.065	0.065
00213	205 lbs.	60 lbs.	0.100	0.109	0.084	0.090			0.069
00226	205 lbs.	60 lbs.	0.045	0.045	0.035				
00232		60 lbs.		0.050	0.053	0.079	0.030	0.069	0.052

* Chronological order of fish consumed is based on 1973 data. The 1974 questionnaire was not completed.

** Sample was depleted during initial analysis, using decachlorobiphenyl techniques. See Appendix (B) for decachlorobiphenyl blood values.

*** Sample lost (broken, not received, etc.)

MONS 056473

TABLE 6. CORRELATION OF FISH EXPOSURE WITH PCB VALUES IN BLOOD OVER A TWO YEAR PERIOD, MANISTEE GROUP

I.D.#	1973 Fish Consumed	1974 Fish Consumed	BLOOD SPECIMENS - Total PCB in parts per million									
			Nov.'73	Jul.'74	10-2-74	10-22-74	Nov.'74	Dec.'74	Jan.'75	Feb.'75	Mar.'75	Jun.'7
0252	0 lbs.	0 lbs.	0.014	0.035								
0251	0 lbs.	0 lbs.	0.011	0.010								
0256	5 lbs.	1 lbs.	0.028	0.026								
0250	<6 lbs.	1 lbs.	0.039									
0253	<6 lbs.	2 lbs.	0.025									
0255	1 lbs.	2 lbs.	0.033	0.041								
0254	5 lbs.	2 lbs.	0.027	0.029								
0262	17 lbs.	13 lbs.	0.047	0.041	**	**		0.048	0.057	0.060	0.042	0.047
0264	24 lbs.	15 lbs.	0.037	0.024				0.025	0.036	0.024	0.021	0.035
0269	40 lbs.	23 lbs.	0.025	0.023	**	0.019	0.020	0.023	0.020	0.020	0.018	0.020
0267	22 lbs.	24 lbs.	0.090	0.083				0.082	0.091	***	0.094	0.093
0273		24 lbs.		0.022				0.027				0.026
0271		24 lbs.		0.019						***		0.016
0268	55 lbs.	25 lbs.	0.062	0.064	0.069	**	**	0.070	0.070	0.077	0.056	0.070
0260*	26 lbs.		0.094									
0261	32 lbs.	28 lbs.	0.050	0.117	0.062			0.072	0.074	0.059	0.063	0.089
0270*	29 lbs.		0.038									
0259	53 lbs.	29 lbs.	0.249	0.259					0.227	0.331	0.244	0.264
0266	27 lbs.	30 lbs.	0.124	0.110				0.097	0.144	0.138	0.128	0.102
0257	30 lbs.	32 lbs.	0.105	0.096	0.133	0.128	**	0.127		0.123	0.133	
0272	35 lbs.	35 lbs.		0.089						0.099		0.085
0265	48 lbs.	45 lbs.	0.151	0.123				0.165	0.145	0.125	0.180	0.127
0274	48 lbs.	48 lbs.		0.239				0.206				0.229
0263	79 lbs.	51 lbs.	0.247	0.167				**	0.185	0.133	0.218	0.162
0258	44 lbs.	64 lbs.	0.128	0.169	**	0.153	0.166	0.151		0.131	0.173	0.148

* Chronological order of fish consumed is based on 1973 data. The 1974 questionnaire was not completed.

** Sample was depleted during initial analysis, using decachlorobiphenyl techniques. See Appendix (8) for decachlorobiphenyl blood values.

*** Sample lost (broken, not received, etc.)

TABLE 7. CORRELATION OF FISH EXPOSURE WITH PCB VALUES IN [illegible],
SOUTH HAVEN AND ALGONAC GROUPS.

SOUTH HAVEN				ALGONAC			
I.D.#	1972 Fish Consumed	1973 Fish Consumed	1973 Blood Specimens Total PCB(ppm)	I.D.#	1972 Fish Consumed	1973 Fish Consumed	1973 Blood Specimens Total PCB(ppm)
)0071	28 lbs.	<3 lbs.	0.027	10029	26 lbs.	11 lbs.	0.023
'0072	38 lbs.	<6 lbs.	0.029	11029	26 lbs.	11 lbs.	0.026
)0073	26 lbs.	<10 lbs.	0.053	10166	13 lbs.	13 lbs.	0.025
)0065	51 lbs.	13 lbs.	0.026	10033	26 lbs.	16 lbs.	0.030
)0066	31 lbs.	17 lbs.	0.035	10130	31 lbs.	20 lbs.	0.019
)0106	35 lbs.	17 lbs.	0.031	10013	26 lbs.	24 lbs.	0.011
)0121	26 lbs.	24 lbs.	0.064	10111	52 lbs.	26 lbs.	0.021
)0105	70 lbs.	24 lbs.	0.045	10171		>26 lbs.	0.017
)0115	23 lbs.	26 lbs.	0.025	10172		≥26 lbs.	0.033
)0070	26 lbs.	26 lbs.	0.068	10129	39 lbs.	28 lbs.	0.030
)0126	42 lbs.	26 lbs.	0.059	10118	52 lbs.	34 lbs.	0.021
)0067	68 lbs.	26 lbs.	0.037	10100	36 lbs.	38 lbs.	0.038
)0111*	26 lbs.		0.040	10035	26 lbs.	42 lbs.	0.012
)0130	30 lbs.	26 lbs.	0.034	10150	48 lbs.	26 lbs.	0.013
)0036	26 lbs.	28 lbs.	0.025	10085*	45 lbs.		0.017
)0124	50 lbs.	28 lbs.	0.056	10110	77 lbs.	57 lbs.	0.020
)0119*	30 lbs.		0.164	10138		52 lbs.	0.032
)0053	50 lbs.	30 lbs.	0.034	10084*	126 lbs.		0.042
)0139*	36 lbs.		0.032	10168		135 lbs.	0.026
)0140*	36 lbs.		0.018				
)0120	24 lbs.	39 lbs.	0.048				
)0055	32 lbs.	40 lbs.	0.326				
)0035	26 lbs.	42 lbs.	0.043				
)0064	43 lbs.	45 lbs.	0.123				
)0104*	48 lbs.		0.056				
)0114	45 lbs.	55 lbs.	0.029				
)0122	60 lbs.	58 lbs.	0.150				
)0100*	63 lbs.		0.035				
)0128	55 lbs.	64 lbs.	0.082				
)0062	98 lbs.	69 lbs.	0.366				
)0116*	80 lbs.		0.118				

* Chronological order of fish consumed is based on 1972 data. The 1973 questionnaire was not completed.

MONS 056475

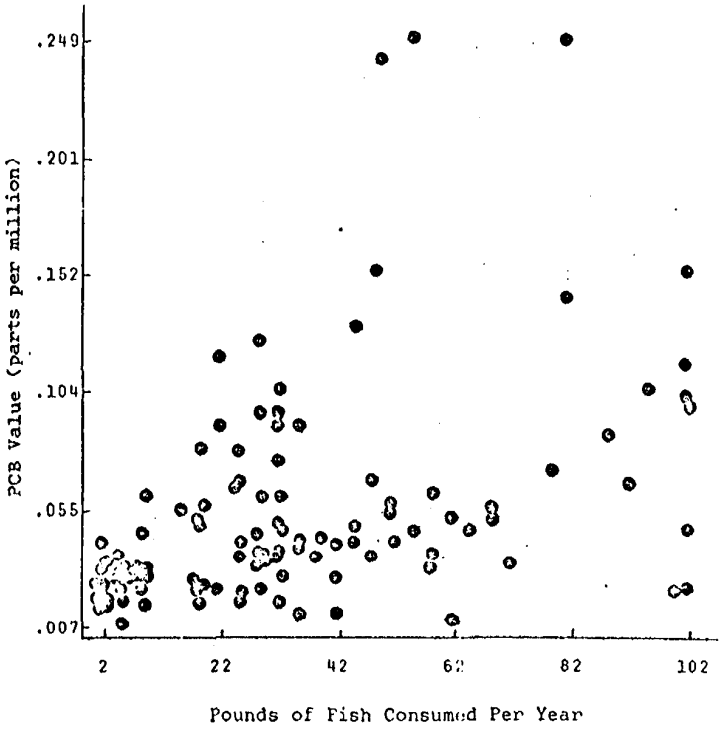


FIGURE 3. CORRELATION OF PCB LEVELS IN HUMAN BLOOD WITH FISH CONSUMPTION

MONS 056476

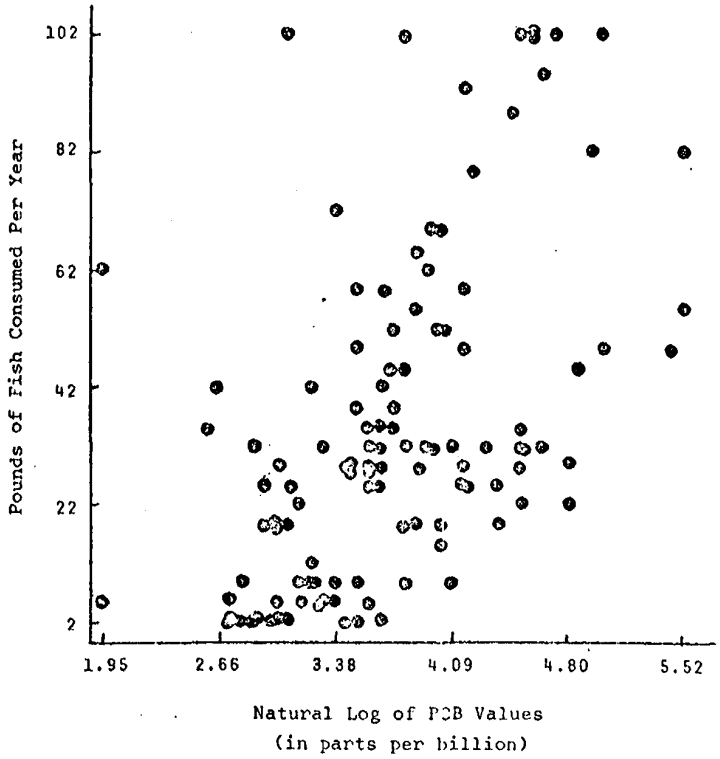


FIGURE 4. SCATTER PLOT OF POUNDS OF FISH CONSUMED v_s THE NATURAL LOG OF PCB LEVELS IN HUMAN BLOOD

MONS 056477

bordering Lake Michigan. The mean level of PCB for participants grouped in the major fish consumption categories is shown for each community in Table 8. These data clearly show that the presence of PCB in human blood increases significantly for persons in each ascending consumption category regardless of geographic location.

Apparently any exposure to contaminated fish causes an elevation of PCB blood levels. The control group included persons who ate no fish and those who ate up to six pounds per year. A comparison of control participants who ate no fish from Lake Michigan with those who ate an occasional fish revealed significantly higher ($p<<.002$) PCB levels for the latter group (Table 9). It has been reported that the general population is exposed to PCB from a variety of sources and this is reflected in detectable levels in blood (17) and adipose (3, 4). The study confirms such a conclusion as shown by the values found in the control subjects. It appears that an additional exposure through consumption of contaminated fish, even in small amounts, contributes to the existing body burden of PCB in humans.

A substantial exposure to contaminated fish makes a highly significant contribution to the PCB body burden. A comparison of PCB blood values for exposed persons (those who consume 24 or more pounds of Lake Michigan fish per year) in the four communities bordering the Lake were compared to the blood values for the zero consumption controls. Using the 1973 baseline data from Tables 4, 5, 6 & 7 the mean PCB values for these two groups were found to be 0.077 ppm ($n = 71$, range 0.025 to 0.366 ppm) and 0.017 ppm ($n = 16$, range 0.007 to 0.042 ppm) respectively. The difference between these mean values is highly significant ($p<<.001$)* indicating a positive correlation between exposure to contaminated fish and the accumulation of PCB in the human body.

*A Cochran adjustment to the t-test was used because of the large difference in the variances of the PCB values for the two groups (18).

MONS 056478

The significantly higher PCB levels found in humans is the direct result of an additional exposure to PCB from eating contaminated fish from Lake Michigan. Participants from Algonac, a community bordering Lake St. Clair on the opposite side of the State, who consumed fish from Lake St. Clair, had a mean PCB blood level of 0.023 ppm (n=14, range 0.011 to 0.042 ppm)(1973 data from Tables 4, 5, 6 & 7). The difference between the mean PCB values for these two exposed groups is highly significant ($p<<.001$).* The mean PCB value for the Algonac fish eaters compares closely with that of the Lake Michigan control group (Table 8). Fish in Lake St. Clair do not contain the high levels of PCB reported for Lake Michigan sport fish. Thus, fish eating per se does not cause the observed elevation of PCB in humans but consumption of the more highly contaminated species in Lake Michigan does.

No annual variation in PCB levels in humans could be demonstrated. The mean PCB values for the control and exposed groups did not appear to change markedly from year to year (1973 baseline v_s 1974 baseline, Table 8). The exposed group included individuals reporting many combinations of size, species (contamination level) and quantities of fish consumed. Considering these individual variations in PCB exposure the similarity of the annual mean values strongly suggest that PCB levels in exposed persons as a group do not change significantly from year to year. The PCB blood values for a selection of participants who maintained their usual fish consumption habits during the study period are individually illustrated in Figures 10 and 11 (Appendix D).

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*A Cochran adjustment of the t-test was used because of the large difference in the variances of the PCB values for the two groups (18).

MONS 056479

TABLE 8. YEAR TO YEAR COMPARISON OF MEAN PCB VALUES BY CITY

City	Mean Total PCB Value (ppm) 1973			Mean Total PCB Value (ppm) 1974		
	Participant Group			Participant Group		
	Control	Inter-mediate	Exposed	Control	Inter-mediate	Exposed
Traverse City	0.017	0.026	0.060	0.019	0.036	0.061
Manistee	0.025	0.068	0.109	0.028	0.029	0.120
Ludington	0.021	0.048	0.062	0.024	0.058	0.055
South Haven	0.028	0.036	0.083			
Algonac	-	0.024	0.023			
Group Mean First 3 Cities	0.020	0.044	0.073	0.023	0.041	0.075

MONS 056480

TABLE 9. EVALUATION OF MEAN PCB VALUES, CONTROL GROUP

FISH CONSUMPTION	MEAN PCB VALUE (ppm) 1973	MEAN PCB VALUE (ppm) 1974
Ate No Fish 0 lbs./year	0.017 n=16	0.016 n=10
Ate Some Fish 1-6 lbs./year	0.024 n=21	0.026 n=15

MONS 056481

No seasonal variation in the level of PCB in humans was observed. Initially it was believed that exposure from fish consumption would follow the seasonal pattern of summer fishing and winter lay off. Examination of the dietary data collected during the study shows that this was not the case. Instead, as discussed previously, fish are consumed throughout the year with the higher rates during the active fishing period and lower rates during the winter when preserved fish from the previous season are eaten. The absence of a seasonal consumption pattern was reflected by the absence of statistically significant changes in PCB blood levels during the year (Tables 4, 5, 6 and Appendix D). The April 1974, November 1973, and July 1974 specimen collections were intended to illustrate blood levels just prior to the resumption of fishing, near the end of fishing, and in the middle of a fishing season respectively. The PCB levels determined for these collections were not significantly different. To test this further an artificially created seasonal comparison was established by comparing blood values for a selection of participants who reportedly ate eight more fish meals over the three month period prior to one specimen collection then in a like period prior to another specimen collection. Fifteen subjects met this arbitrary criteria. A paired t-test of the differences in blood PCB for the high and low consumption periods was not significant ($t = 0.99$).

Abstinence from Lake Michigan fish consumption did not significantly change the level of PCB in the blood of persons in the exposed group. Forty-four participants from Traverse City, Manistee, and Ludington were selected and asked to abstain from consumption of Lake Michigan fish at the conclusion of the 1974 fishing season. Thirty-six persons were able to maintain an abstinence period of 90 days or more. The 1974 annual consumption, length of abstinence, and PCB blood levels at various times during abstinence for these participants are shown in Table 10. The length of abstinence

MONS 056482

Table 10. Correlation of PCB Levels in Humans and Length of Abstinence from Fish Consumption

Participant No.	Annual Fish Consumption lbs/year *	Period of Abstinence Dates	#Days	Total PCB Concentrations in Blood (ppm) Number of Days After Termination of Fish Consumption 0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270
Ludington																						
00218	10	12-74/3-75	85	.026		.029				.026												
00215	15	11-74/3-75	127				.063		.059			.064										
[illegible]	19	12-74/3-75	85	[illegible]		[illegible]				.052												
00229	26	11-74/4-75	158				.019		.021			.017			.021							
00228	27	12-74/4-75	126	.033			.022			.017		.021										
00210	33	12-74/4-75	121	.085		.070				.070		.084										
00211	39	12-74/4-75	121	lost		.066				.066		.065										
Manistee																						
00262	13	12-74/3-75	108	.048		.057			.060		.042											
00264	15	10-75/3-75	147					.025		.036		.024		.021								
00269	23	10-75/3-75	176	**		.020			.023		.020		.020			.018						
00267	24	12-74/6-75	176	.082		.091		**			.094					.093						
00263	25	10-74/3-75	176	.069		**			.070		.070		.077			.056						
00261	28	12-74/3-75	108	.072		.074			.059		.063											
00266	30	12-74/6-75	176	.097		.144			.138		.128					.102						
00257	32	10-74/3-75	148	**			.127				.123			.133								
00265	45	11-74/2-75	87		.165			.143		.125												
00263	51	11-74/3-75	124		**			.185		.133		.218										
Traverse City																						
00332	8	1-75/5-75	131	.051						.040			.061									
00333	8	1-75/5-75	131	.038						.032			.031									
00366	9	11-74/4-75	152					.021		.016				.019								
00355	18	11-74/5-75	160					.052		.044					.043							
00352	30	1-75/5-75	138	.090			.097			.086			.130									
00353	30	1-75/5-75	138	.074			.070			.054			.066									
00325	31	1-75/4-75	90	.058		.052				.074												
00395	34	12-74/5-75	153		.013		.019				.013			.025								
00370	36	9-74/5-75	249								.036		.041				.056			.032		
00302	41	12-74/5-75	153		.056		.050				.058			.044								
00326	44	1-75/4-75	90	.069		.057				.049												
00342	44	8-74/5-75	278										.031			.036			.027			.032
00312	47	1-75/5-75	139	.062		.067				.045			.061									
00330	56	12-74/4-75	104		.040		.045				.036											
00358	79	12-74/5-75	153		.177		.152				.135			.147								
00331	90	12-74/5-25	104		.101		.140				.121											
00355	120	11-74/5-75	193					.067		.073				.059			.068					
00339	152	12-75/3-75	151		.086		.088							.082								
00338	162	1-75/5-75	141	.078		.081				.095			.088									

* Based on 1974 report

** Sample was depleted using decachlorobiphenyl techniques. See Appendix (B) for decachlorobiphenyl values.

MONS 056483

ranged from 85 days to 278 days. The date of initiation of abstinence was staggered because of the individuality of the end of the fishing season. In the same manner the end of the abstinence period was staggered and determined by the beginning of the 1975 fishing season or the desire of individuals to avoid wasting their store of frozen catch from the previous season. As discussed previously, this portion of the study represented a change in the normal habits of most of these subjects, a matter which took considerable self restraint.

An examination of the blood levels over the abstaining period (Table 10) shows variation but no pronounced decline in PCB for the group. In fact, more subjects showed no change or a rise than showed a decline in PCB level over time. PCB blood values for selected subjects who abstained from eating fish for part of the study period are individually illustrated in Figures 12 and 13 (Appendix E). An analysis of variance for 24 subjects with a beginning blood PCB level of at least 0.040 ppm showed no significant decline in levels over time. A paired t-test using the initial and final abstaining blood values showed an average net increase in PCB and no statistical significance in the recorded change during abstinence from Lake Michigan fish. It must be concluded that abstinence from eating contaminated fish did not reduce the PCB blood levels in these subjects. Thus, an evaluation of the half-life of the concentration of PCB's in humans could not be made within an abstinence period of 90 to 278 days.

Cooked Lake Michigan fish consumed by study participants contained PCB's but at levels lower than those reported for raw whole fish. Participants were asked to record their fish consumption in dietary logs and save a portion of their fish meals for analysis. Questionnaire and dietary log data were used to estimate the annual fish consumption reported in Tables 4, 5, 6 & 7. This data also

MONS 056484

indicated that Lake Trout, Steelhead and Salmon (Coho or Chinook) represented the perferred and most frequently consumed species of fish for most of the participants. An exception was found in South Haven where chubs and perch were eaten more frequently. The PCB levels found in cooked fish collected from participants during 1973 and 1974 are shown in Table 11. This data has been averaged and arranged by fish category and community in Table 12. These results represent tests on composites of collected samples of Lake Michigan fish eaten by individuals during the study. The PCB level in cooked Lake Trout ranged from 1.03 to 4.67 ppm, in cooked salmon from 0.48 to 5.38 ppm, and in other cooked fish from 0.36 to 2.06 ppm. These levels are decidedly lower than the level of PCB contamination generally reported for raw whole Trout and Salmon (Table 1). This is not unexpected since preparation (trimming away fatty tissue) and cooking (loss of fat drippings)* have been shown to decrease the concentration of PCB's (19). The results indicate that actual human exposure to PCB is less than that expected from data for raw whole fish.

The calculated quantity of PCB ingested by eating Lake Michigan fish averaged 46.5 mg/year and ranged from 14.17 to 114.31 mg/year for 91 of the exposed group participants (Table 13). PCB ingestion for each individual was determined by proportioning the reported annual fish consumption by frequency of species eaten and the cooked fish PCB levels (Table 11) for these fish. The community average (Table 12) was used for instances where cooked fish determinations were not available for a participant. Because fish consumption was found to vary from year to year the average annual consumption for the two baseline years of the study was used in each case.

*Tests on two samples of cooking oil used for deep fat frying of fish showed 0.84 and 0.76 ppm PCB.

MONS 056485

TABLE 11. PCB LEVELS IN COOKED FISH CONSUMED BY HOBBLES

PARTICIPANT I.D.#	PCB 1254 CONCENTRATION (parts per million)					
	LAKE TROUT		SALMON		OTHER*	
	1973	1974	1973	1974	1973	1974
00210	4.51	3.24		1.39		
00213			0.48	0.96		
00214		5.17	1.85			
00217	2.83		3.86	3.66		
00223	3.53		3.01		0.76	
00225	1.63		2.96		1.46	
00227	4.00		3.48			
00231		3.65		4.63		0.86
00219	3.68		1.87		0.98	
00257	3.75	2.84		2.81		
00259	1.03	1.48	2.99			
00261	1.87	4.38	3.34			
00263			4.89		0.90	0.62
00266	2.48	2.07		1.32		
00268		1.97		3.18		0.56
00270	5.13				1.05	
00273		1.69		1.49		
00300	3.66	4.17			1.81	1.66
00308	3.44				1.69	
00309	1.58	2.35	3.18			1.17
00311			2.54			
00312	3.17	2.43		2.84		0.36
00323	2.23	1.69			0.73	
00325	1.63	1.63	2.64	4.11	0.73	
00327						
00328	4.67	1.66		2.75	1.56	
00330	2.84	2.31		1.29		0.97
00332	1.04	1.83		4.63		0.63
00334	3.47					
00336	3.18				2.06	1.52
00339		2.32				1.98
00350				3.45		0.56
00365		1.85		5.38		
00035	3.28		2.18		1.87	
00062	3.48					
00104			3.33			
00105			2.99			
00114						
00119	5.63		1.45			
00120	4.81		2.72		0.47	
00121	5.62		2.39			
00130	1.84					

*Includes: Pike, Whitefish, Smelt, Chubs, Menominee and Perch

MONS 056486

TABLE 12. COOKED FISH SURVEY, MEAN PCB CONCENTRATIONS BY COMMUNITY

LOCATION	FISH SPECIES								
	LAKE TROUT			SALMON			OTHER*		
	PCB 1254 (ppm)			PCB 1254 (ppm)			PCB 1254 (ppm)		
	1973	1974	1973-74	1973	1974	1973-74	1973	1974	1973-74
Traverse City:									
Mean	2.80	2.22	2.52	2.78	3.49	3.28	1.43	1.11	1.25
No. Samples	11	10	21	3	7	10	6	8	14
Manistee:									
Mean	2.85	2.40	2.60	3.74	2.20	2.86	0.98	0.59	0.78
No. Samples	5	6	11	3	4	7	2	2	4
Ludington:									
Mean	3.36	4.02	3.58	2.50	2.65	2.55	1.06	0.86	0.99
No. Samples	6	3	9	7	4	11	3	1	4
South Haven:									
Mean	4.11			2.51			1.17		
No. Samples	6			6			2		

*Includes: Pike, Whitefish, Smelt, Chubs, Menominee and Perch

MONS 056487

Table 13 shows that PCB ingestion depends not only on the quantity of fish eaten but also on the species and contamination level of the fish which compose the meals. Thus a person consuming a given quantity of fish could receive a varying amount of PCB depending on the species and the size of fish eaten. In general, PCB ingestion corresponded with the quantity of fish consumed.

The correlation between PCB ingestion from fish and human PCB blood levels was significant. An annual PCB dose was determined for each participant listed in Table 13 and compared to the corresponding mean (1973-74 baseline) PCB blood levels as illustrated in Figures 5 (Traverse City group) and 6 (Manistee, Ludington and South Haven groups). The correlation coefficients for these plots are 0.347 and 0.316 respectively, indicating significance at the 5% probability level. The dose-blood level relationship confirms the pounds of fish consumed - blood level relationship shown previously in Figure 3 and Table 8. Interpretation of the meaning of these correlations requires caution because of the number of uncontrolled variables which complicates accurate estimation of PCB ingestion. The PCB level in each fish eaten, the exact number of pounds consumed annually, the species and quantity of fish consumed in previous years, and the conditions controlling the appearance and level of PCB in human blood are all factors which contribute to the high degree of scatter seen in the data plots.

The maximum allowable ingestion recommended for protracted exposure to PCB is being exceeded by the majority of the exposed group participants. It has been estimated that 1 µg/Kg body weight/day represents the maximum allowable dose for protracted ingestion of PCB's (8). This was based on extrapolation from the lowest total dose (500 mg) reported to produce an effect in man and the assumption that PCB exposure may be tolerated over a long period if daily exposure is held to minimal levels. The calculated mean daily dose

MDNS 056488

TABLE 13. ESTIMATED PCB INGESTION FROM FISH, ANNUAL DOSE AND MEAN BLOOD VALUES FOR PERSONS CONSUMING 24 OR MORE POUNDS PER YEAR

I.D.#	Body Weight Kg	1973-75 Mean Annual Fish Consumption Kg/yr.	Calculated Annual* PCB Ingestion From Fish mg PCB/yr.	Annual PCB Dose mg/Kg/yr.	Mean Blood** PCB Value (ppm)
TRAVERSE CITY:					
00306	79	11.3	14.17	0.18	0.040
00363	77	11.8	23.85	0.30	0.040
00337	97	12.2	24.77	0.26	0.041
00324	82	12.7	18.65	0.23	0.050
00355	75	12.7	22.33	0.28	0.043
00353	64	13.2	37.14	0.59	0.056
00352	74	13.6	38.43	0.52	0.098
00334	79	14.1	36.31†	0.48	0.047
00325	64	14.5	33.82†	0.53	0.070
00335	61	14.5	37.48	0.61	0.042
00369	57	15.0	30.01	0.53	0.036
00336	77	15.9	31.09†	0.40	0.037
00365	75	15.9	23.66†	0.32	0.015
00370	82	16.3	46.11	0.68	0.034
00309	100	17.0	28.01†	0.28	0.048
00326	71	17.2	40.16	0.57	0.052
00351	61	19.1	26.97	0.44	0.081
00301	70	20.4	35.57	0.51	0.038
00372	54	20.4	35.57	0.67	0.045
00362	70	20.8	69.30	0.99	0.034
00312	59	21.8	29.09†	0.49	0.065
00359	136	21.8	38.28	0.28	0.033
00300	64	23.1	40.65†	0.64	0.060
00304	57	23.1	40.65	0.71	0.057
00322	66	23.1	65.33	0.99	0.068
00361	70	24.0	48.37	0.68	0.065
00328	64	26.3	78.98†	1.24	0.048
00330	54	28.1	45.39†	0.83	0.036
00323	75	30.8	44.65†	0.60	0.054
00358	73	35.8	44.79	0.62	0.131
00356	100	44.5	78.15	0.78	0.064
00364	77	47.2	94.91	1.23	0.009

* Values for PCB concentrations in fish provided from tests on cooked composite samples provided by participants or from community averages for such samples.

† Determined from tests on fish samples provided by the participants

** Average of the November 1973 and July 1974 blood values

MONS 056489

TABLE 13. (continued)

I.D.#	Body Weight Kg	1973-75 Mean Annual Fish Consumption Kg/yr.	Calculated Annual* PCB Ingestion From Fish mg PCB/yr.	Annual PCB Dose mg/Kg/yr.	Mean Blood** PCB Value (ppm)
TRAVERSE CITY (continued)					
00350	84	48.5	75.23†	0.90	0.106
00373	68	50.8	89.22	1.31	0.064
00339	63	53.9	90.57†	1.44	0.078
00311	102	57.2	100.47†	0.99	0.072
00331	82	57.2	92.27	1.13	0.143
00338	81	36.1	102.80	1.25	0.117
MANISTEE					
00273	82	10.9	17.53†	0.22	0.022
00271	69	10.9	29.43	0.42	0.019
00268	75	11.1	44.53†	0.60	0.062
00260	65	11.8	23.02	0.35	0.094
00261	91	13.6	35.68†	0.39	0.084
00270	73	13.2	45.52†	0.63	0.038
00266	76	13.2	25.00†	0.33	0.110
00257	66	14.1	43.64†	0.66	0.096
00269	104	14.5	35.62	0.34	0.023
00272	84	15.9	42.93	0.51	0.089
00259	94	18.6	36.29†	0.38	0.259
00265	104	21.3	74.42	0.71	0.137
00274	70	21.8	35.05	0.50	0.239
00263	106	29.5	103.67†	0.98	0.207
00258	82	24.5	76.03	0.93	0.148
LUDINGTON					
00217	94	10.9	36.88†	0.39	0.071
00223	79	11.8	29.65†	0.37	0.036
00229	57	12.5	24.02	0.42	0.021
00216	67	12.3	30.60	0.46	0.031
00228	102	12.3	23.79	0.23	0.031
00231	64	12.7	51.34†	0.81	0.031
00210	68	12.9	37.18†	0.54	0.076

* Values for PCB concentrations in fish provided from tests on cooked composite samples provided by participants or from community averages for such samples.

† Determined from tests on fish samples provided by the participant

** Average of the November 1973 and July 1974 blood values

MONS 056490

TABLE 13. (continued)

I.D.#	Body Weight Kg	1973-75 Mean Annual Fish Consumption Kg/yr.	Calculated Annual* PCB Ingestion From Fish mg PCB/yr.	Annual PCB Dose mg/Kg/yr.	Mean Blood** PCB Value (ppm)
LUDINGTON (continued)					
00211	64	14.3	40.99	0.64	0.077
00232	93	27.2	109.01	1.18	0.107
00226	70	60.1	49.76	0.70	0.045
00213	81	60.1	78.29†	0.96	0.050
SOUTH HAVEN					
00066	52	10.9	18.56	0.36	0.035
00115	52	11.3	35.72	0.69	0.025
00121	61	11.3	41.75†	0.68	0.064
00070	74	11.8	29.16	0.40	0.068
00106	73	11.8	31.87	0.44	0.031
00111	91	11.8	27.66	0.31	0.040
00036	57	12.2	29.80	0.53	0.025
00130	73	12.7	28.48†	0.39	0.034
00119	84	13.6	42.49†	0.51	0.164
00065	75	14.5	24.75	0.33	0.026
00120	61	14.5	57.68†	0.94	0.048
00035	75	15.4	37.53†	0.50	0.043
00126	61	15.4	26.31	0.43	0.059
00055	74	16.3	56.66	0.76	0.326
00140	68	16.3	38.36	0.56	0.018
00139	55	17.2	40.44	0.73	0.032
00124	57	17.7	41.50	0.73	0.056
00053	89	18.1	42.56	0.48	0.034
00064	79	19.9	46.82	0.59	0.123
00067	82	21.3	36.37	0.46	0.037
00105	95	21.3	57.61†	0.61	0.045
00104	79	21.8	44.29†	0.56	0.056
00114	82	22.7	71.45†	0.88	0.029
00122	101	26.8	45.66	0.45	0.150
00128	86	27.2	46.44	0.54	0.082
00116	79	36.3	114.31	1.44	0.118
00062	84	38.1	84.58†	1.01	0.366

* Values for PCB concentrations in fish provided from tests on cooked composite samples provided by participants or from community averages for such samples.

† Determined from tests on fish samples provided by the participant

** Average of the November 1973 and July 1974 blood values

MONS 056491

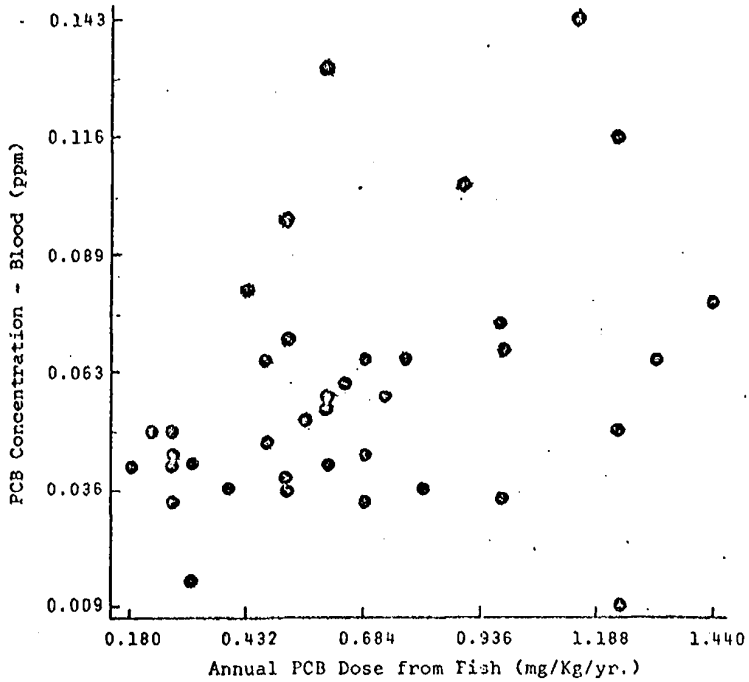

FIGURE 5. CORRELATION OF PCB LEVELS IN HUMAN BLOOD WITH PCB INGESTION FROM FISH, TRAVERSE CITY GROUP

MONS 056492

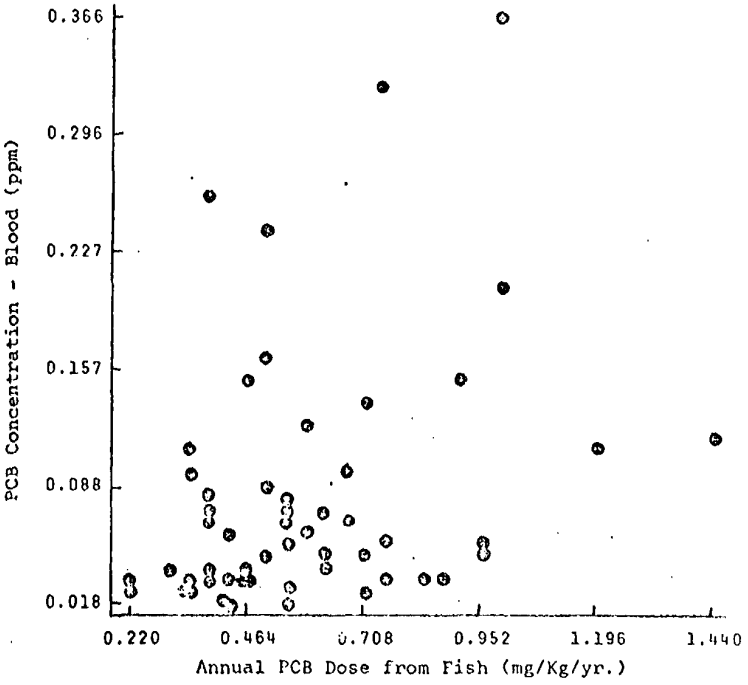

FIGURE 6. CORRELATION OF PCB LEVELS IN HUMAN BLOOD WITH PCB INGESTION FROM FISH, MANISTEE, LUDINGTON, AND SOUTH HAVEN GROUPS

MONS 056493

received by the exposed group listed in Table 13 is 1.70 µg/Kg/day and ranges from 0.49 to 3.94 µg/Kg/day (Table 14). Except for those in the lower range these levels clearly exceed the recommended dose established for long term continuous exposure.

Lake Michigan fish consumption has been shown to be intermittently continuous through the year. This provides repeated doses of PCB which appear sufficient to maintain a PCB equilibrium within humans. Sport fishing has become increasingly popular in the past eight years and activity is expected to continue. Consumption of salmonids from the Great Lakes is and will continue to represent a source of prolonged exposure to PCB. If the average annual rate of PCB ingestion from fish shown in Table 14 is continued over the years, and if net accumulation occurs, the average fish eater could receive a total PCB dose equal to the 200 mg safety limit in 4.3 years. The lowest total dose reported to produce an overt effect in humans (500 mg) would be reached in 10.7 years. An individual who habitually consumed large quantities of contaminated fish would reach these total dose levels sooner, 1.75 years and 4.3 years respectively if the top range value is used. Such a person would reach the average total dose received by those with Yusho symptoms (2,000 mg) in 17.5 years. If it is proven that such total dose accumulations actually occur in persons who consume significant quantities of contaminated Great Lakes fish, then the long term exposure to PCB contaminated fish could prove important.

Segregation of exposed participants on the basis of reported prior history of fish consumption revealed that PCB blood levels rise with the length of time fish have been consumed (Table 15). All of the groups received comparable PCB doses during 1973-74 yet the mean blood PCB levels for this period were directly related to the extent of prior fish consumption. Higher blood levels were associated with a longer history of consuming Lake Michigan fish. Although the details and quantities of prior fish consumption are not

MONS 056494

TABLE 14. CALCULATED PCB INGESTION AND DOSE FROM CONSUMPTION OF GREAT LAKES FISH*

PCB Ingestion			PCB Dose		
	Annual mg/year	Daily µg/day		Annual mg/Kg/year	Daily µg/Kg/day
Mean	46.500	0.130	Mean	0.620	1.700
Range: low	14.170	0.038	Range: low	0.180	0.490
high	114.310	0.313	high	1.440	3.940

*Calculated from data on 91 exposed subjects in Table 13.

MONS 056495

TABLE 15. CORRELATION OF BLOOD PCB LEVELS WITH NUMBER OF YEARS OF FISH CONSUMPTION

No. Years Lake Michigan Fish Eaten	Mean PCB Dose mg/Kg/year	Mean Blood PCB (ppm)	No. Subjects*
1 - 3	0.58	0.046	3
4 - 6	0.62	0.055	35
7 - 9	0.53	0.068	6
10 or more	0.59	0.082	43

*From Traverse City, Manistee, Ludington and South Haven

MONS 056496

known the data strongly suggest that long term exposure to PCB contaminated fish results in a net accumulation of PCB in human tissue. Furthermore, the absence of any data indicating a decline in PCB levels suggests that a PCB equilibrium may exist and that recirculation of PCB may be occurring in humans. This would cause target organs to be continuously exposed to PCB for years.

Exposed and control participants were asked if they had experienced any of 17 health conditions. Many of the conditions described the signs and symptoms reported for Yusho disease. There was no significant difference between the number of exposed and control persons responding "yes" to any condition (Table 16). No health conditions or group of symptoms could be identified that were clearly related to PCB exposure. This implies that exposure to PCB from eating contaminated fish and the presence of PCB in exposed persons had not caused an observable acute effect in humans at the time of the study. This does not exclude the possibility that effects too subtle for detection by our survey are occurring.

The blood PCB levels for those responding "yes" to a health condition were not significantly different from those responding "no" to the condition (Table 17). Standard t-tests and a Behrens-Fisher technique for unequal variances were used to evaluate the data. A significant number of exposed subjects or subjects with high PCB levels would be expected to report a given health condition if such a condition was a manifestation of the presence of PCB in humans. The failure of this to occur suggests that there was no common illness related to PCB exposure or that PCB blood levels were not related to the appearance of any of the conditions investigated.

MONS 056497

TABLE 16. EXPOSED AND CONTROL PARTICIPANT RESPONSES TO HEALTH CONDITIONS

Health Conditions	Fish Eater		Non-Fish Eater		Fisher's Probability
	Yes	No	Yes	No	
1. Stomach pains or vomitting for more than one week.	1	75	1	37	0.56
2. Kidney and/or bladder problems.	4	72	2	36	0.68
3. Stroke.	0	76	0	38	--
4. General Fatigue	7	69	2	36	0.37
5. Persistent headaches.	5	71	1	37	0.35
6. Weakness or loss of movement in any part of body.	1	75	1	37	0.56
7. Difficulty with coordination.	2	74	0	38	0.44
8. Fainting, dizzy spells or loss of consciousness.	5	71	1	37	0.35
9. Numbness or tingling in arms or legs.	5	71	0	38	0.13
10. Loss of memory (for names or numbers).	0	76	0	38	--
11. Shaking, unsteadiness, especially of hands and arms.	1	75	0	38	0.67
12. Problems with speech or writing.	0	76	0	38	--
13. Convulsions or a seizure	0	76	0	38	--
14. Persistent blurred vision or unusual sensitivity to light.	3	73	0	38	0.29
15. Discharge from eyes or swelling of the eyelids.	1	75	1	37	0.56
16. Persistent skin eruptions or unusual skin pigmentation or itching.	1	75	0	38	0.67
17. Increased perspiration of palms.	1	75	0	38	0.67
18. Other	7	69	0	38	0.05
Any of the above (at least one).	24	52	7	31	0.10

*Fisher's exact test of the difference between "yes" proportions for the two groups.

MONS 056498

TABLE 17. MEAN AND STANDARD DEVIATIONS OF PCB VALUES AND SAMPLE SIZES FOR EACH CONDITION BY REPORTING STATUS

Condition	Reported Having Condition			Reported Not Having Condition		
	Mean of PCB Values (ppm)	Standard Deviation of PCB Values (ppm)	Sample Size	Mean of PCB Values (ppm)	Standard Deviation of PCB Values (ppm)	Sample Size
1. Stomach	0.058	0.055	7	0.049	0.043	123
2. Kidney/Bladder	0.036	0.020	14	0.051	0.045	116
3. Stroke	--	--	1*	--	00	129
4. Fatigue	0.042**	0.026	14	0.051	0.045	116
5. Headaches	0.047	0.051	19	0.050	0.042	111
6. Weakness/Loss of Movement	0.056	0.034	8	0.049	0.044	122
7. Coordination	0.041	0.048	2	0.050	0.044	128
8. Fainting/Dizziness	0.036	0.029	12	0.051	0.045	118
9. Numbness/Tingling	0.047**	0.028	15	0.050	0.045	115
10. Loss of Memory	0.015**	0.006	4	0.051	0.044	126
11. Shaking/Unsteadiness	0.051	0.036	4	0.050	0.044	126
12. Speech/Writing	--	--	1*	--	--	129
13. Convulsions	--	--	0*	--	--	130
14. Blurred Vision	0.088**	0.102	4	0.049	0.041	126
15. Eye Discharge	0.047	0.024	6	0.050	0.044	124
16. Skin	0.057	0.032	8	0.049	0.044	122
17. Palm Perspiration	0.028	0.009	2	0.050	0.044	128
18. Other	0.067**	0.077	14	0.048	0.038	116
19. At Least One of Above	0.051	0.046	64	0.049	0.041	66

* No test possible; only one or no cases.

** Unequal variances. These were tested using a standard F-ratio test.

MONS 056499

RESIDUE ANALYTICAL METHODOLOGY:

The development of a suitable method for detection and quantitation of PCB's in human blood involved the following investigations: (1) extraction, (2) separation from organochlorine pesticides, (3) derivation, and (4) gas chromatography. Practicality of the analytical scheme, time involved per sample, and reliability of data obtained were important factors in the choice of procedures. Extraction, separation by silica gel chromatography and direct GLC determination of Aroclors (PCB's) requires about half as much laboratory time as the combination of extraction, separation by chemical degradation, perchlorination, and GLC determination of decachlorobiphenyl. At best, either scheme can provide only a good estimate of the degree of exposure of the individual to the PCB compounds actually present in the sample. Accumulation of PCB's in the biological system appears to be higher in the case of pentachlorobiphenyl and more highly chlorinated biphenyls (20).

An extraction procedure for pesticide residue analysis of blood developed specifically for Community Pesticide Study programs (21) was investigated initially. Burke (22) questioned whether the two-hour extraction with hexane on the rotary mixer is efficient. Trial runs indicated less than 45% of Aroclor 1260 was recovered from sheep plasma fortified at the 100 ppb level. Some residue analysts had pretreated blood samples with 60% sulfuric acid to release pesticide residues bound to protein sites (23). Our preliminary investigation of this method produced about 55% recovery of Aroclor 1260 from spiked sheep plasma.

The procedure currently used for extracting PCB's from blood samples incorporates a triple solvent system (i.e., methanol, ethyl ether and hexane), is highly reproducible, and is more quantitative. Since PCB's are fat soluble chemicals

MONS 056500

and the lipids in blood exist mainly as conjugates with protein (24), a method designed to disrupt lipoprotein complexes was required for efficient extraction of blood lipids and, hence, PCB's. Plasma proteins are denatured with methanol, and lipids are extracted with a mixture of ethyl ether and hexane. Recoveries of PCB's (as Aroclors) greater than 88% have been achieved, and a better match of the profiles for extracted Aroclors with those for reference standards have been observed with this method.

Separation of PCB's from organochlorine pesticides is necessary with either direct analysis or derivation in order to reduce or eliminate interference by DDT and its isomers. Although numerous separation techniques have been reported in scientific journals, efforts were focused on charcoal column chromatography, chemical degradation of organochlorine pesticides, and silica gel column chromatography. These are more suited to detecting the small quantities of PCB found in human blood samples than macro methods such as the silicic acid-Celite column chromatography (25).

The charcoal column technique described by Berg et al. (26) appeared to meet our requirement for handling micro quantities of PCB's (5 micrograms or less). A 6 mm i.d. Chromaflex column (Kontes) is plugged with glass wool and filled with 1.1 gram Fisher No. 5-690 charcoal as an acetone slurry. An acetone solution of the sample is added on the column. The DDT group pesticides are eluted with 90 ml of Solvent A (25% acetone in ethyl ether) and PCB's with 60 ml of benzene. Under these conditons, some PCB's eluted in the first fraction but increasing the weight of charcoal from 1.1 gram to 2.5 grams reduced this effect. Although the separation of PCB's appeared to be satisfactory (80%), the elution required as much as three hours per sample, probably due to the formation of vapor pockets.

A micro scale procedure for the quantitative separation of PCB's from DDT and its analogs was studied in a cooperative effort by Dr. Frotter (27). Certain organochlorine pesticides

are dehydrochlorinated with ethanolic potassium hydroxide to their respective olefins (28) which are then oxidized by chromic acid in acetic acid to dichlorobenzophenone (29). The latter is separated from PCB's by Florisil column chromatography. Trotter (30) achieved recoveries greater than 80% in the ppm range (based on a 5-gram sample) and recoveries of 60% or more in the ppb range for Aroclor 1254 and 1260. Chemical degradation has little effect on certain pesticides such as dieldrin, oxychlordane and Mirex which may not be at levels sufficient to interfere with the quantitation of PCB's in human blood. Our chemical degradation experiments with 0.5 µg to 1.0 µg amounts of Arochlor 1254 and 1260 indicated that recoveries of 65-90% can be achieved.

In an effort to seek a more simplified and less time consuming technique, attention was turned to the use of a micro column of silica gel (31). Woelm silica gel, activity grade I, deactivated with 3% (v/w) distilled water, was sandwiched between layers of anhydrous sodium sulfate in a 7 mm i.d. Chromaflex column. This column was tested with standard solutions of Aroclor 1254 (2.0 µg) and organochlorine pesticides by elution with hexane. With the exception of p,p' DDE, pesticides were found to be completely separated from Aroclor 1254 (about 95% recovery). However, the presence of DDE did not interfere with direct analysis of PCB's. Elution of the PCB fraction can be accomplished in 20-25 minutes.

Perchlorination of PCB's to decachlorobiphenyl (DCB) with antimony pentachloride (32) was evaluated for improved sensitivity of PCB analysis. Conversion of a multicomponent residue to a single compound offered several advantages. This procedure would provide a qualitative and quantitative confirmatory method for PCB's. Also, it would minimize the necessary analytical judgments involved in the measurement of gas chromatographic tracings of a multicomponent residue. Considerable effort was devoted to adapting this procedure for

MONS 056502

routine analysis of PCB's in human blood samples, and several problems became evident. At less than 50 ppb PCB levels, conversions were less reproducible and reagent blanks became significant, e.g., 2-10 ppb DCB for a 5-gram sample. Antimony pentachloride has been found to contain a bromide contaminant which produced bromononachlorobiphenyl during perchlorination (33), but several lots of a high purity grade (99.99%) received in this laboratory did not show any evidence of this contaminant.

The suggestion that PCB levels in blood samples can be determined directly (27) appeared to be more practical for this survey because the gas chromatograph response can be adjusted to permit detection levels as low as 10 ppb total PCB's (Aroclor 1254 and 1260) and fewer steps would be involved in preparing samples for gas chromatographic analysis. The GLC column used for the determination of individual Aroclors was 4%SE-30/6%OV-210. Estimation of PCB residue levels in sample extracts was based on the heights of five peaks in comparison with respective peaks derived from reference Aroclors. The GLC peak having a relative retention time of 1.26 (p,p' DDE=1.00; T-203°C) on this column was distinct and was selected for the estimation of Aroclor 1254 levels.

The analytical procedure incorporating extraction, separation by silica gel chromatography, and direct determination by gas chromatography was the final method of choice primarily because of its superior analytical productivity rate. The output by this procedure was approximately 4 samples per man-day, whereas only 2 samples per man-day could be processed according to the analytical scheme comprising extraction, spearation by chemical degradation, perchlorination, and GLC determination of decachlorobiphenyl. The analyst had less confidence in the latter procedure as yields of the derivative were variable, particularly at the low PCB levels often found

MONS 056503

in blood samples. Also at low residue levels, reagent blanks contributed a significant proportion of the decachlorobiphenyl product and were not always dependable.

CHOSEN ANALYTICAL PROCEDURE:

I. Reagents:

A. Solvents: Hexane, ethyl ether and methanol suitable for use in pesticide residue analysis or distilled-in-glass (Burdick and Jackson Laboratories, Inc. 1953 S. Harvey St., Muskegon, Mi. 49442).

B. Silica gel: Woelm, activity grade I (ICN Pharmaceuticals, Inc.). Deactivate with 3% (v/w) distilled water and store in tightly sealed glass container.

C. Sodium sulfate: Anhydrous, granular (Mallinckrodt No. 8024 or equivalent). Store at 130° in glass stoppered bottle.

D. Antimony pentachloride: Anhydrous, 99.99% (Research Organic/Inorganic Chemical Corp., No. SB-03).

E. Reference standards: Aroclor 1254 (FDA 370) and decachlorobiphenyl (FDA 1056) obtained from Division of Chemical Technology, Bureau of Foods, FDA, Washington, D.C.; Aroclor 1260 from P.T.S.E.L., U.S.E.P.A., Research Triagle Park, N.C.

II. Apparatus:

A. Chromatographic column; Chromaflex glass column, 7 mm i.d. (Kontes No. K-420100-0022). Prepare columns by plugging with small wad of clean glass wool, filling to depth of 1 cm with granular anhydrous Na_2SO_4, adding 3.0 grams absorbent, and topping with another 2-3 cm layer of Na_2SO_4.

MONS 056504

B. Concentrating apparatus: Evaporative concentrator complete with modified micro Snyder column, joint 19/22 (Kontes No. K-569250); concentrator tubes sizes 1025 and 2525 (Kontes No. K-570050).

C. Gas chromatograph: Tracor Model MT220 with tritium foil parallel-plate design electron capture detectors; glass, U-shaped columns, 6' x 4 mm i.d.

1. Pack first column with 4%SE-30/6%OV-210 on 80-100 mesh Gas Chrom Q.

2. Pack second column with 5%OV-210 on 80-100 mesh Supelcoport.

3. Pack third column with 1%OV-101 on 100-120 mesh Gas Chrom Q.

4. Columns 1 and 2 are used for direct analysis of PCB's and Column 3 for decachlorobiphenyl.

5. Operating conditions: Nitrogen flow, 100 ml/min. (Columns 1 and 3) and 40 ml/min. (Column 2); column and detector temperatures, 203°C; injector temperature, 225°C.

III. Extraction:

Transfer 5.0 ml plasma or serum into clean culture tube (20 x 150 mm) having Teflon-lined screw cap. Add 4 ml methanol and swirl on Vortex mixer about 10 seconds. Add 5 ml hexane-ethyl ether (1:1) and attach screw cap securely. Mix contents 10 minutes, using rotary mixer at 50-55 rpm. Centrifuge for 2-5 minutes at 2000 rpm. Transfer upper layer to 25 ml concentrator tube. Repeat extraction twice with 5 ml hexane-ethyl ether. Add carborundum chip, attach micro Snyder column and concentrate extract to about 0.5 ml using a water bath at 80-90°C.

MONS 056505

IV. Separation on Silica Gel:

Wash silica gel chromatographic column with 5 ml hexane. Just as the last of the hexane enters sodium sulfate layer, quantitatively transfer concentrated extract to the top of the column using a disposable glass pipet. Elute PCB's from column with 19 ml hexane. Collect a first fraction, 4 ml, and a second fraction, 15 ml, in graduated centrifuge tubes. Discard the first fraction. The second fraction contains PCB's and p,p' DDE. Reduce volume to 1.0 ml with a slight stream of nitrogen directed upon hexane solution. If transferred to a 10-ml concentrator tube, the extract may be reduced to 0.5 ml.

V. Gas Chromatographic Analysis:

Inject a 5-8 µl aliquot of concentrated eluate, and, if necessary, adjust to suitable volume. Heights of peaks having the following relative retention times (p,p' DDE=1.00) are measured and compared with respective peaks for reference standards of Aroclor 1254 and 1260: 1.26; 1.42; 1.69; 1.85 and 2.62 (4%SE-30/6%OV-210 column at 203°C). Aroclor 1254 is calculated on the basis of peak 1.26. Peak 1.86 is considered to be derived from Aroclor 1260. After subtracting apparent Aroclor 1254 portions, i.e., peak heights, of peaks 1.42, 1.69 and 2.62, the mean Aroclor 1260 level of these peaks is then calculated. No corrections were made to reflect 100% recovery of PCB's. The limit of detection is 10 ppb Aroclor 1254 and 6 ppb Aroclor 1260.

VI. Controls and Reagent Blanks:

Each batch of 12 samples consists of one control and one reagent blank. Quality control samples are prepared by fortifying sheep plasma with ethyl acetate solutions of Aroclor 1254 (66-132 ppb) and Aroclor 1260 (16-30 ppb). Recoveries were 91% Aroclor 1254 (range 80-105%) and 85% Aroclor 1260 (range 75-96%). PCB's were not detected in sheep plasma blanks used in this study.

MONS 056506

VII. Confirmation by Perchlorination:

The procedure by Armour (32) was followed for qualitative confirmation of PCB's in certain blood samples.

VIII. Precision:

Sixty-seven blood samples were analyzed in duplicates by the direct analysis procedure. The total levels for the first reading were correlated with those of the second reading as a test of reliability (Figure 7). The correlation coefficient was 0.972 which indicates a highly significant agreement for the replicate tests and a high degree of reliability of analytical data obtained in this study.

SPECIAL TESTS:

I. Figures 8 and 9 are illustrations of the chromatograms of hexane extracts following chemical degradation of samples containing Aroclor 1254 and 1260 respectively. Plot B in Figure 8 represents a sample of cooking oil used by one of the study subjects for fish preparation. As shown the PCB found in the cooking oil closely resembles the GLC tracing for the Aroclor 1254 standard (plot A Figure 8). The absence of pronounced peaks indicative of Aroclor 1260 in the oil used to cook Lake Michigan fish and the Aroclor concentrations found in the blood of participants (Appendix A) indicate that those who consume Lake Michigan fish are primarily exposed to the Aroclor 1254 form of PCB.

II. Best Specimen:

Fasting and nonfasting blood specimens were collected from subjects in the PCB Study and an investigation involving a related chemical, polybrominated biphenyl (PBB). There

MONS 056507

was no significant difference in the chlorinated or brominated biphenyl concentrations for the two types of blood specimens (Table 18). Thus either type of sample would be satisfactory for the detection of PCB.

III. Breast Milk Specimen:

Traverse City participant 00324 who consumed an average of 28 pounds of Lake Michigan fish during 1973-74 gave birth to a child in January of 1975. A breast milk specimen collected during January contained 4 ppm PCB, Aroclor 1254 (fat basis - the specimen had 2% fat content). In contrast, a blood specimen collected in January contained 0.053 ppm total PCB. This shows that body tissues with a significant lipid content will contain PCB's which are fat soluble at levels greater than those found in blood. It also suggests that pregnant or nursing women should consume Lake Michigan fish with caution.

MONS 056508

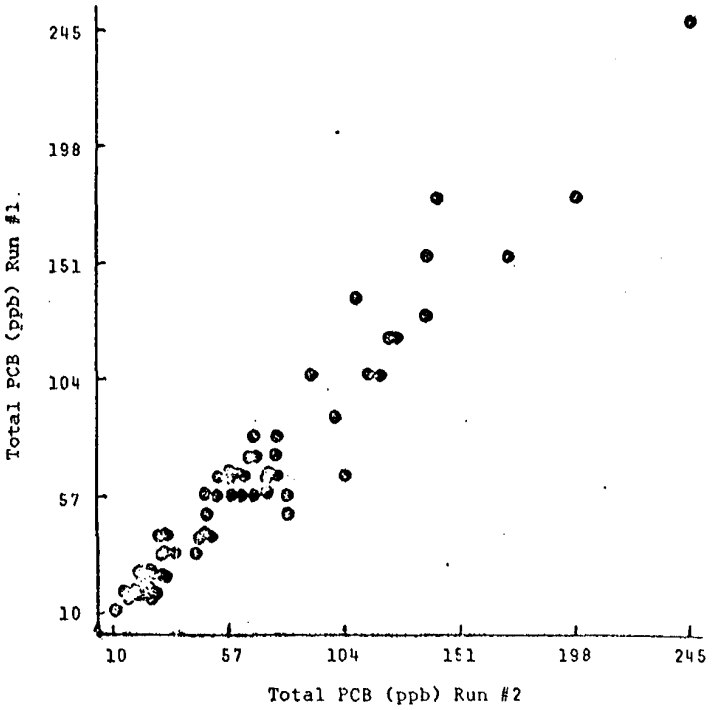

FIGURE 7. ANALYTICAL PRECISION, PLOT OF PCB VALUES FOR REPLICATE TESTS.

MONS 056509

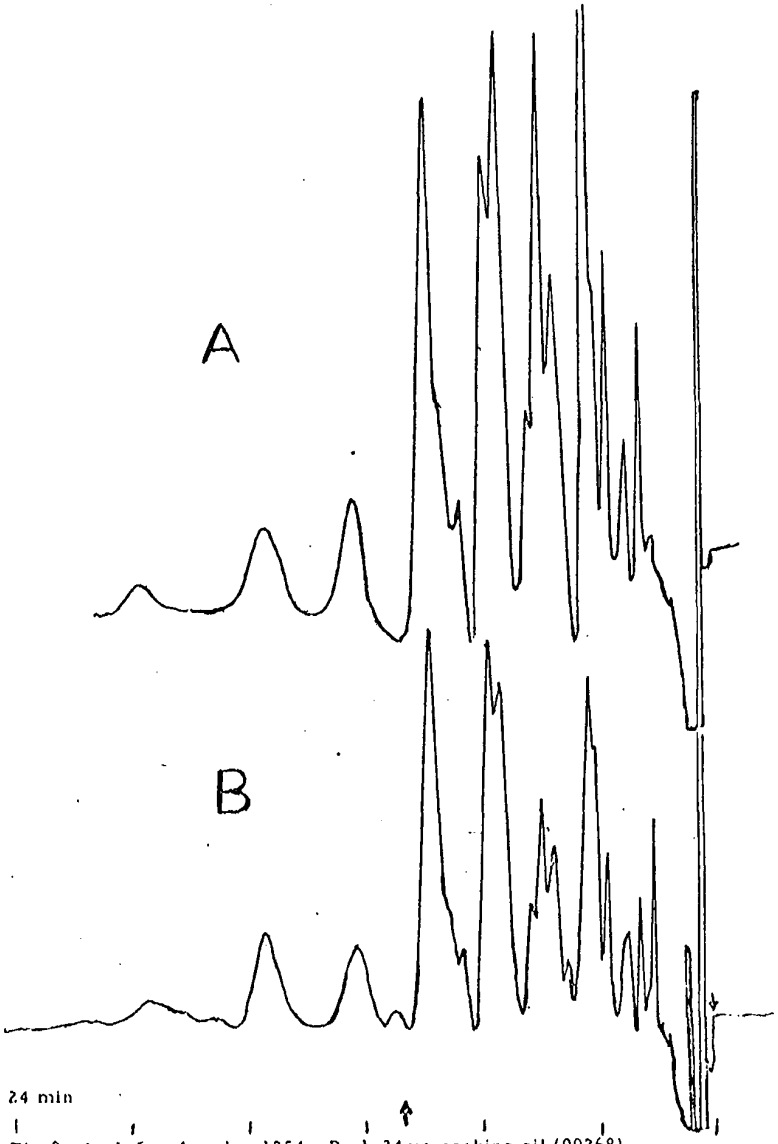

Fig. 8. A, 1.5ng Aroclor 1254. B, 1.34mg cooking oil (00268). GLC parameters: see Fig. 9.

MONS 056510

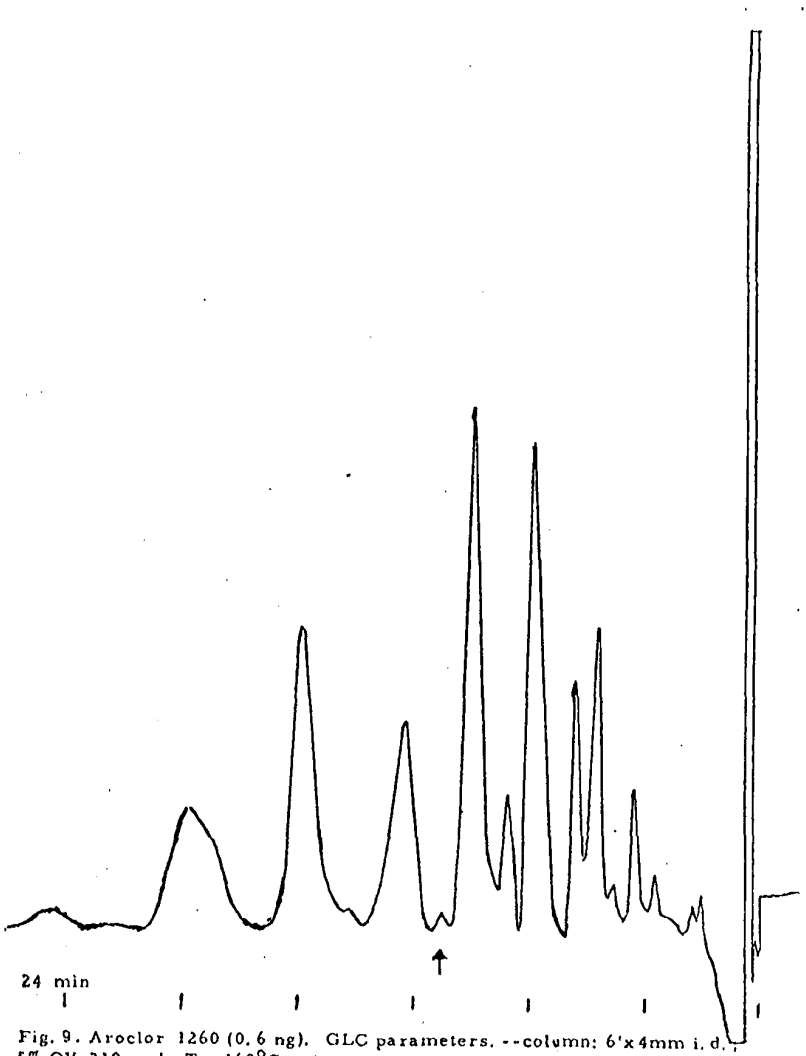

Fig. 9. Aroclor 1260 (0.6 ng). GLC parameters. --column: 6'x4mm i.d. 5% OV-210; col. T.: 168°C. Arrow: p,p' DDT elution position. Flow: 70ml N_2/min.

MONS 056511

TABLE 18. BEST SPECIMEN COMPARISON

HALOGENATED BIPHENYL VALUE (ppm)	
Fasting Blood	Nonfasting Blood
0.015	0.017
0.051	0.050
0.001	0.001
0.001	0.001
0.066	0.065
0.035	0.038
0.088	0.102
0.005	0.005
0.022	0.025
0.042	0.040
0.025	0.025

MONS 056512

A P P E N D I X A

FINAL LABORATORY DATA FOR PCB TESTS ON BLOOD SPECIMENS COLLECTED DURING THE STUDY

MONS 056513

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00035	29-10-73	0.030	0.013	0.043
00036	29-10-73	0.020	0.005	0.025
00053	29-10-73	0.024	0.010	0.034
00055	29-10-73	0.218	0.108	0.326
00062	27-10-73	0.255	0.111	0.366
00064	27-10-73	0.091	0.032	0.123
00065	29-10-73	0.018	0.008	0.026
00066	29-10-73	0.027	0.008	0.035
00067	29-10-73	0.027	0.010	0.037
00070	29-10-73	0.045	0.023	0.068
00071	27-10-73	0.022	0.005	0.027
00072	29-10-73	0.022	0.007	0.029
00073	29-10-73	0.038	0.015	0.053
00100	29-10-73	0.027	0.008	0.035
00104	29-10-73	0.042	0.014	0.056
00105	29-10-73	0.032	0.013	0.045
00106	29-10-73	0.025	0.006	0.031
00111	29-10-73	0.033	0.007	0.040
00114	29-10-73	0.019	0.010	0.029
00115	29-10-73	0.018	0.007	0.025
00116	29-10-73	0.075	0.043	0.118
00119	29-10-73	0.126	0.038	0.164
00120	29-10-73	0.040	0.008	0.048
00121	29-10-73	0.049	0.015	0.064
00122	29-10-73	0.116	0.034	0.150
00124	29-10-73	0.036	0.020	0.056
00126	29-10-73	0.048	0.011	0.059
00128	29-10-73	0.064	0.018	0.082
00130	29-10-73	0.025	0.009	0.034
00139	29-10-73	0.023	0.009	0.032
00140	29-10-73	0.012	0.006	0.018
00200	01-11-73	0.027	0.010	0.037
00200	11-07-74	0.033	0.009	0.042
00201	01-11-73	0.023	0.006	0.029
00201	11-07-74	0.026	0.005	0.031
00202	01-11-73	0.012	0.003	0.015
00202	29-07-74	0.015	0.003	0.018
00203	01-11-73	0.009	0.002	0.011
00203	11-07-74	0.011	0.003	0.014
00204	01-11-73	0.015	0.002	0.017
00205	01-11-73	0.015	0.005	0.020
00205	11-07-74	0.022	0.007	0.029
00206	01-11-73	0.016	0.005	0.021
00206	11-07-74	0.016	0.005	0.021
00207	01-11-73	0.014	0.004	0.018
00208	01-11-73	0.022	0.008	0.030
00209	01-11-73	0.012	0.004	0.016
00209	29-07-74	0.012	0.004	0.016
00210	01-11-73	0.048	0.017	0.065
00210	11-07-74	0.057	0.019	0.076
00210	18-12-74	0.065	0.020	0.085
00210	21-01-75	0.052	0.018	0.070
00210	10-03-75	0.053	0.017	0.070
00210	15-04-75	0.064	0.020	0.084
00210	21-05-75	0.063	0.021	0.084
00211	01-11-73	0.050	0.015	0.065
00211	11-07-74	0.060	0.017	0.077
00211	21-01-75	0.052	0.014	0.066
00211	10-03-75	0.050	0.016	0.066
00211	15-04-75	0.049	0.016	0.065
00211	21-05-75	0.048	0.018	0.066
00212	01-11-73	0.022	0.004	0.026
00213	01-11-73	0.068	0.032	0.100
00213	11-07-74	0.081	0.028	0.109
00213	18-12-74	0.062	0.022	0.084
00213	22-01-75	0.066	0.024	0.090
00213	21-05-75	0.047	0.019	0.066
00214	01-11-73	0.066	0.023	0.089
00214	30-07-74	0.050	0.016	0.066
00214	19-12-74	0.051	0.018	0.069
00215	21-11-73	0.050	0.012	0.062
00215	11-07-74	0.066	0.012	0.078
00215	19-12-74	0.052	0.011	0.063
00215	21-01-75	0.047	0.012	0.059
00215	12-03-75	0.052	0.012	0.064
00215	20-05-75	0.045	0.010	0.055
00216	01-11-73	0.023	0.008	0.031
00217	01-11-73	0.042	0.018	0.060
00217	29-07-74	0.047	0.024	0.071
00217	18-12-74	0.034	0.014	0.048
00217	21-01-75	0.029	0.018	0.047
00217	10-03-75	0.035	0.017	0.052
00217	20-05-75	0.043	0.021	0.064
00218	01-11-75	0.015	0.008	0.023
00218	29-07-74	0.015	0.007	0.022
00218	18-12-74	0.017	0.009	0.026
00218	21-01-75	0.021	0.008	0.029
00218	10-03-75	0.018	0.008	0.026
00219	20-05-75	0.015	0.008	0.023
00219	01-11-73	0.038	0.008	0.046
00220	01-11-73	0.012	0.004	0.016
00221	01-11-73	0.017	0.006	0.023
00222	01-11-73	0.047	0.016	0.063
00223	01-11-73	0.026	0.010	0.036
00224	01-11-73	0.088	0.030	0.118
00225	01-11-73	0.039	0.015	0.054
00226	01-11-73	0.035	0.010	0.045
00226	11-07-74	0.036	0.009	0.045
00226	18-12-74	0.027	0.008	0.035
00227	01-11-73	0.055	0.024	0.079

MONS 056514

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00228	11-07-74	0.025	0.006	0.031
00228	18-12-74	0.027	0.006	0.033
00228	22-01-75	0.019	0.003	0.022
00228	12-03-75	0.014	0.003	0.017
00228	21-04-75	0.016	0.005	0.021
00229	11-07-74	0.016	0.005	0.021
00229	18-12-74	0.015	0.004	0.019
00229	22-01-75	0.016	0.005	0.021
00229	12-03-75	0.012	0.005	0.017
00229	15-04-75	0.016	0.005	0.021
00229	21-05-75	0.017	0.006	0.023
00230	11-07-74	0.040	0.015	0.055
00231	11-07-74	0.023	0.008	0.031
00231	17-12-74	0.020	0.006	0.026
00231	21-01-75	0.019	0.006	0.025
00231	12-03-75	0.043	0.010	0.053
00231	16-04-75	0.033	0.013	0.046
00231	20-05-75	0.018	0.007	0.025
00232	11-07-74	0.039	0.011	0.050
00232	17-12-74	0.042	0.011	0.053
00232	21-01-75	0.062	0.017	0.079
00232	12-03-75	0.022	0.008	0.030
00232	16-04-75	0.052	0.017	0.069
00232	20-05-75	0.039	0.013	0.052
00250	02-11-73	0.029	0.010	0.039
00251	02-11-73	0.009	0.002	0.011
00251	15-07-74	0.007	0.003	0.010
00252	02-11-73	0.009	0.005	0.014
00252	15-07-74	0.023	0.012	0.035
00253	02-11-73	0.020	0.005	0.025
00254	02-11-73	0.023	0.004	0.027
00254	15-07-74	0.025	0.004	0.029
00255	02-11-73	0.026	0.007	0.033
00255	31-07-74	0.031	0.010	0.041
00256	02-11-73	0.018	0.010	0.028
00256	15-07-74	0.015	0.011	0.026
00257	02-11-73	0.087	0.018	0.105
00257	15-07-74	0.075	0.021	0.096
00257	02-10-74	0.107	0.026	0.133
00257	22-10-74	0.103	0.025	0.128
00257	13-12-74	0.105	0.022	0.127
00257	19-02-75	0.102	0.021	0.123
00257	26-03-75	0.107	0.026	0.133
00258	02-11-73	0.100	0.028	0.128
00258	15-07-74	0.130	0.039	0.169
00258	22-10-74	0.116	0.037	0.153
00258	05-11-74	0.130	0.036	0.166
00258	19-02-75	0.104	0.027	0.131
00258	26-03-75	0.138	0.035	0.173
00258	04-06-75	0.108	0.040	0.148
00259	02-11-73	0.179	0.070	0.249
00259	15-07-74	0.193	0.066	0.259
00259	15-01-75	0.153	0.074	0.227
00259	19-02-75	0.244	0.087	0.331
00259	26-03-75	0.164	0.080	0.244
00259	04-06-75	0.170	0.094	0.264
00260	02-11-73	0.067	0.027	0.094
00261	02-11-73	0.036	0.014	0.050
00261	15-07-74	0.081	0.036	0.117
00261	02-10-74	0.043	0.019	0.062
00261	13-12-74	0.053	0.019	0.072
00261	15-01-75	0.054	0.020	0.074
00261	19-02-75	0.042	0.017	0.059
00261	26-03-75	0.045	0.018	0.063
00261	04-06-75	0.066	0.023	0.089
00262	02-11-73	0.035	0.012	0.047
00262	15-07-74	0.030	0.011	0.041
00262	13-12-74	0.036	0.012	0.048
00262	15-01-75	0.045	0.012	0.057
00262	19-02-75	0.044	0.016	0.060
00262	26-03-75	0.032	0.010	0.042
00262	04-06-75	0.036	0.011	0.047
00263	02-11-73	0.185	0.062	0.247
00263	15-07-74	0.121	0.046	0.167
00263	15-01-75	0.139	0.046	0.185
00263	19-02-75	0.095	0.038	0.133
00263	26-03-75	0.152	0.066	0.218
00263	04-06-75	0.115	0.047	0 162
00264	02-11-73	0.027	0.010	0 037
00264	15-07-74	0.016	0.008	0 024
00264	11-12-74	0.018	0.007	0.025
00264	15-01-75	0.028	0.008	0.036
00264	19-02-75	0.016	0.008	0 024
00264	26-03-75	0.014	0.007	0.021
00264	04-06-75	0.022	0.013	0.035
00265	02-11-73	0.119	0.032	0.151
00265	15-07-74	0.091	0.032	0.123
00265	13-12-74	0.128	0.037	0.165
00265	15-01-75	0.112	0.033	0.145
00265	19-02-75	0.096	0.029	0.125
00265	26-03-75	0.140	0.040	0.180
00265	04-06-75	0.096	0.031	0.127
00266	02-11-73	0.092	0.032	0.124
00266	31-07-74	0.081	0.029	0.110
00266	13-12-74	0.076	0.021	0.097
00266	15-01-75	0.111	0.033	0.144
00266	19-02-75	0.104	0.034	0.138
00266	26-03-75	0.094	0.034	0.128
00266	04-06-75	0.074	0.028	0.102
00267	02-11-73	0.065	0.025	0.090

MONS 056515

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00267	31-07-74	0.062	0.021	0.083
00267	11-12-74	0.058	0.024	0.082
00267	15-01-75	0.063	0.028	0.091
00267	26-03-75	0.069	0.025	0.094
00267	04-06-75	0.068	0.025	0.093
00268	02-11-73	0.050	0.012	0.062
00268	15-07-74	0.048	0.016	0.064
00268	02-10-74	0.053	0.016	0.069
00268	11-12-74	0.054	0.016	0.070
00268	15-01-75	0.055	0.015	0.070
00268	19-02-75	0.062	0.015	0.077
00268	26-03-75	0.044	0.012	0.056
00268	04-06-75	0.057	0.013	0.070
00269	02-11-73	0.023	0.002	0.025
00269	15-07-74	0.020	0.003	0.023
00269	22-10-74	0.017	0.002	0.019
00269	05-11-74	0.018	0.002	0.020
00269	11-12-74	0.020	0.003	0.023
00269	15-01-75	0.018	0.002	0.020
00269	19-02-75	0.018	0.002	0.020
00269	26-03-75	0.016	0.002	0.018
00269	04-06-75	0.016	0.004	0.020
00270	02-11-73	0.026	0.012	0.038
00271	15-07-74	0.015	0.004	0.019
00271	04-06-75	0.012	0.004	0.016
00272	31-07-74	0.072	0.017	0.089
00272	19-02-75	0.077	0.022	0.099
00272	04-06-75	0.060	0.025	0.085
00273	15-07-74	0.016	0.006	0.022
00273	11-12-74	0.021	0.006	0.027
00273	04-06-75	0.019	0.007	0.026
00274	15-07-74	0.142	0.097	0.239
00274	11-12-74	0.120	0.086	0.206
00274	04-06-75	0.130	0.099	0.229
00300	13-11-73	0.051	0.009	0.060
00301	13-11-73	0.034	0.004	0.038
00301	24-04-74	0.023	0.005	0.028
00301	10-01-75	0.028	0.005	0.033
00301	12-02-75	0.023	0.003	0.026
00302	13-11-73	0.071	0.014	0.085
00302	10-01-75	0.043	0.013	0.056
00302	12-02-75	0.039	0.011	0.050
00302	09-04-75	0.044	0.014	0.058
00302	28-05-75	0.034	0.010	0.044
00303	10-01-75	0.032	0.011	0.043
00304	13-11-73	0.045	0.012	0.057
00305	10-01-75	0.011	0.002	0.013
00305	12-02-75	0.016	0.003	0.019
00305	09-04-75	0.010	0.003	0.013
00305	28-05-75	0.014	0.011	0.025
00306	13-11-73	0.031	0.009	0.040
00307	13-11-73	0.018	0.004	0.022
00309	13-11-73	0.029	0.009	0.038
00309	16-07-74	0.046	0.012	0.058
00309	09-01-75	0.046	0.012	0.058
00309	27-05-75	0.055	0.014	0.069
00310	13-11-73	0.017	0.003	0.020
00310	16-07-74	0.019	0.004	0.023
00310	10-01-75	0.022	0.004	0.026
00310	27-05-75	0.017	0.004	0.021
00311	13-11-73	0.073	0.023	0.096
00311	24-04-74	0.062	0.021	0.083
00311	16-07-74	0.050	0.018	0.068
00312	13-11-73	0.046	0.019	0.065
00312	01-08-74	0.046	0.018	0.064
00312	09-01-75	0.048	0.014	0.062
00312	12-02-75	0.029	0.018	0.047
00312	28-05-75	0.038	0.023	0.061
00312	09-04-75	0.029	0.016	0.045
00313	13-11-73	0.006	0.001	0.007
00313	01-08-74	0.009	0.001	0.010
00314	13-11-73	0.010	0.001	0.011
00315	13-11-73	0.028	0.011	0.039
00315	01-08-74	0.030	0.011	0.041
00316	13-11-73	0.013	0.003	0.016
00316	16-07-74	0.008	0.003	0.011
00317	13-11-73	0.020	0.004	0.024
00317	16-07-74	0.023	0.006	0.029
00318	13-11-73	0.006	0.004	0.010
00318	16-07-74	0.008	0.004	0.012
00319	13-11-73	0.009	0.004	0.013
00319	16-07-74	0.010	0.005	0.015
00320	13-11-73	0.010	0.004	0.014
00320	16-07-74	0.010	0.005	0.015
00321	13-11-73	0.027	0.005	0.032
00322	13-11-73	0.040	0.015	0.055
00322	16-07-74	0.061	0.019	0.080
00322	09-01-75	0.060	0.017	0.077
00323	13-11-73	0.035	0.015	0.050
00323	01-08-74	0.040	0.018	0.058
00323	09-01-75	0.055	0.023	0.078
00323	27-05-75	0.054	0.017	0.071
00324	13-11-73	0.033	0.008	0.041
00324	01-08-74	0.046	0.012	0.058
00324	09-01-75	0.044	0.009	0.053
00324	27-05-75	0.029	0.010	0.039
00325	13-11-75	0.058	0.017	0.075
00325	01-08-74	0.049	0.016	0.065
00325	09-01-75	0.044	0.014	0.058
00325	12-02-75	0.034	0.018	0.052

MONS 056516

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00355	16-07-74	0.032	0.011	0.043
00355	09-01-75	0.040	0.012	0.052
00355	12-02-75	0.033	0.011	0.044
00355	09-04-75	0.033	0.010	0.043
00355	28-05-75	0.135	0.038	0.173
00356	16-07-74	0.047	0.017	0.064
00356	09-01-75	0.048	0.019	0.067
00356	12-02-75	0.053	0.020	0.073
00356	24-04-74	0.051	0.020	0.071
00356	09-04-75	0.043	0.016	0.059
00356	28-05-75	0.049	0.019	0.068
00357	24-04-74	0.015	0.007	0.022
00357	16-07-74	0.018	0.008	0.026
00357	09-01-75	0.013	0.008	0.021
00357	20-05-75	0.019	0.009	0.028
00358	24-04-74	0.100	0.042	0.142
00358	16-07-74	0.091	0.040	0.131
00358	09-01-75	0.119	0.058	0.177
00358	12-02-75	0.108	0.044	0.152
00358	09-04-75	0.097	0.038	0.135
00358	28-05-75	0.106	0.041	0.147
00359	24-04-74	0.026	0.007	0.033
00359	01-08-74	0.025	0.008	0.033
00360	24-04-74	0.017	0.002	0.019
00360	01-08-74	0.020	0.003	0.023
00361	24-04-74	0.031	0.012	0.043
00361	24-04-74	0.028	0.011	0.039
00361	17-07-74	0.045	0.020	0.065
00361	10-01-75	0.028	0.012	0.040
00361	12-02-75	0.029	0.013	0.042
00362	24-04-74	0.025	0.009	0.034
00362	16-07-74	0.025	0.009	0.034
00362	09-01-75	0.024	0.007	0.031
00362	12-02-75	0.027	0.009	0.036
00362	09-04-75	0.019	0.008	0.027
00362	28-05-75	0.025	0.007	0.032
00363	24-04-74	0.026	0.010	0.036
00363	01-08-74	0.040	0.021	0.061
00363	12-02-75	0.025	0.014	0.039
00363	09-04-75	0.021	0.011	0.032
00363	27-05-75	0.025	0.015	0.040
00364	24-04-74	0.006	0.001	0.007
00364	01-08-74	0.008	0.003	0.011
00364	28-05-75	0.012	0.002	0.014
00365	24-04-74	0.011	0.003	0.014
00365	16-07-74	0.011	0.004	0.015
00365	10-01-75	0.008	0.004	0.012
00365	12-02-75	0.007	0.005	0.012
00365	09-04-75	0.010	0.003	0.013
00365	28-05-75	0.009	0.004	0.013
00366	24-04-74	0.015	0.003	0.018
00366	16-07-74	0.020	0.003	0.023
00366	10-01-75	0.016	0.005	0.021
00366	12-02-75	0.014	0.002	0.016
00366	09-04-75	0.016	0.003	0.019
00367	16-07-74	0.057	0.036	0.093
00367	10-01-75	0.074	0.049	0.123
00367	28-05-75	0.057	0.029	0.086
00368	16-07-74	0.013	0.010	0.023
00369	17-07-74	0.028	0.008	0.036
00369	10-01-75	0.027	0.009	0.036
00369	12-02-75	0.027	0.007	0.034
00370	01-08-74	0.023	0.011	0.034
00370	10-01-75	0.023	0.013	0.036
00370	11-02-75	0.030	0.011	0.041
00370	08-01-75	0.036	0.020	0.056
00370	27-05-75	0.022	0.010	0.032
00371	01-08-74	0.018	0.007	0.025
00371	10-01-75	0.028	0.008	0.036
00371	11-02-75	0.019	0.006	0.025
00371	08-04-75	0.019	0.007	0.026
00371	27-05-75	0.028	0.009	0.037
00372	01-08-74	0.031	0.014	0.045
00373	01-08-74	0.040	0.024	0.064
00373	09-01-75	0.049	0.027	0.076
00373	12-02-75	0.036	0.023	0.059
00373	09-04-75	0.041	0.023	0.064
00373	28-05-75	0.044	0.024	0.068
00374	10-01-75	0.040	0.005	0.045
00374	12-02-75	0.037	0.004	0.041
00374	09-04-75	0.039	0.004	0.043
10013	07-11-73	0.007	0.004	0.011
10029	07-11-73	0.020	0.003	0.023
10033	07-11-73	0.023	0.007	0.030
10035	07-11-73	0.009	0.003	0.012
10084	07-11-73	0.032	0.010	0.042
10085	07-11-73	0.013	0.004	0.017
10100	07-11-73	0.029	0.009	0.038
10110	07-11-73	0.016	0.004	0.020
10111	07-11-73	0.018	0.003	0.021
10118	07-11-73	0.016	0.005	0.021
10129	07-11-73	0.022	0.008	0.030
10130	07-11-73	0.012	0.007	0.019
10138	07-11-73	0.024	0.008	0.032
10150	07-11-73	0.009	0.004	0.013
10166	07-11-73	0.021	0.004	0.025
10168	07-11-73	0.020	0.006	0.026
10171	07-11-73	0.014	0.003	0.017
10172	07-11-73	0.024	0.009	0.033
11029	07-11-73	0.018	0.008	0.026

MONS 056517

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00325	09-04-75	0.056	0.018	0.074
00325	28-05-75	0.051	0.014	0.065
00326	13-11-73	0.033	0.011	0.044
00326	01-08-74	0.044	0.015	0.059
00326	09-01-75	0.053	0.016	0.069
00326	12-02-75	0.041	0.016	0.057
00326	09-04-75	0.035	0.014	0.049
00326	28-05-75	0.056	0.018	0.074
00327	13-11-73	0.017	0.005	0.022
00328	13-11-73	0.032	0.015	0.047
00328	24-04-74	0.025	0.012	0.037
00328	16-07-74	0.033	0.015	0.048
00328	09-01-75	0.042	0.016	0.058
00328	12-02-75	0.039	0.017	0.056
00328	27-05-75	0.027	0.015	0.042
00329	13-11-73	0.017	0.005	0.022
00330	13-11-73	0.020	0.010	0.030
00330	24-04-74	0.031	0.013	0.044
00330	16-07-74	0.030	0.012	0.042
00330	10-01-75	0.028	0.012	0.040
00330	12-02-75	0.034	0.011	0.045
00330	09-04-75	0.025	0.011	0.036
00330	28-05-75	0.031	0.014	0.045
00331	13-11-73	0.108	0.044	0.152
00331	24-04-74	0.083	0.032	0.115
00331	16-07-74	0.098	0.036	0.134
00331	10-01-75	0.075	0.026	0.101
00331	12-02-75	0.098	0.042	0.140
00331	09-04-75	0.085	0.036	0.121
00331	28-05-75	0.121	0.051	0.172
00332	09-01-75	0.035	0.016	0.051
00332	09-04-75	0.027	0.013	0.040
00332	28-05-75	0.044	0.017	0.061
00333	09-01-75	0.029	0.009	0.038
00333	09-04-75	0.025	0.007	0.032
00333	28-05-75	0.024	0.007	0.031
00334	13-11-73	0.029	0.011	0.040
00334	01-08-74	0.039	0.014	0.053
00334	12-02-75	0.036	0.015	0.051
00334	27-05-75	0.038	0.014	0.052
00335	13-11-73	0.035	0.008	0.043
00335	01-08-74	0.033	0.008	0.041
00335	12-02-75	0.044	0.012	0.056
00335	09-04-75	0.053	0.013	0.066
00335	27-05-75	0.063	0.014	0.077
00336	13-11-73	0.025	0.012	0.037
00336	16-07-74	0.024	0.013	0.037
00336	12-02-75	0.041	0.017	0.058
00336	09-04-75	0.019	0.010	0.029
00336	27-05-75	0.024	0.010	0.034

ID#	Collection Date	parts per million PCB 1254	PCB 1260	PCB Total
00337	13-11-73	0.023	0.005	0.028
00337	16-07-74	0.031	0.010	0.041
00337	12-02-75	0.033	0.007	0.040
00337	09-04-75	0.032	0.010	0.042
00337	27-05-75	0.032	0.007	0.039
00338	13-11-73	0.081	0.033	0.114
00338	01-08-74	0.079	0.041	0.120
00038	09-01-75	0.052	0.026	0.078
00338	12-02-75	0.052	0.029	0.081
00338	09-04-75	0.061	0.034	0.095
00338	27-05-75	0.060	0.028	0.088
00339	13-11-73	0.058	0.025	0.083
00339	16-07-74	0.054	0.018	0.072
00339	09-01-75	0.061	0.025	0.086
00339	12-02-75	0.063	0.025	0.088
00339	28-05-75	0.061	0.021	0.082
00340	13-11-73	0.034	0.007	0.041
00341	13-11-73	0.024	0.003	0.027
00342	13-11-73	0.011	0.007	0.018
00342	01-08-74	0.013	0.009	0.022
00343	13-11-73	0.010	0.004	0.014
00343	16-07-74	0.014	0.005	0.019
00344	13-11-73	0.009	0.006	0.015
00345	13-11-73	0.015	0.004	0.019
00346	13-11-73	0.013	0.004	0.017
00346	16-07-74	0.014	0.005	0.019
00347	13-11-73	0.012	0.003	0.015
00347	16-07-74	0.018	0.004	0.022
00348	13-11-73	0.033	0.012	0.045
00349	13-11-73	0.016	0.004	0.020
00350	09-01-75	0.074	0.032	0.106
00350	28-05-75	0.088	0.033	0.121
00351	13-11-73	0.059	0.022	0.081
00351	09-01-75	0.061	0.019	0.080
00351	28-05-75	0.068	0.024	0.092
00352	13-11-73	0.070	0.023	0.093
00352	16-07-74	0.074	0.029	0.103
00352	10-01-75	0.068	0.022	0.090
00352	19-02-75	0.072	0.025	0.097
00352	16-04-75	0.062	0.024	0.086
00352	27-05-75	0.095	0.035	0.130
00353	13-11-73	0.038	0.010	0.048
00353	16-07-74	0.048	0.015	0.063
00353	10-01-75	0.058	0.016	0.074
00353	19-02-75	0.053	0.017	0.070
00353	16-04-75	0.043	0.011	0.054
00353	27-05-75	0.052	0.014	0.066
00354	13-11-73	0.030	0.003	0.033
00354	01-08-74	0.023	0.004	0.027
00355	24-04-74	0.033	0.008	0.041

MONS 056518

A P P E N D I X B

LABORATORY DATA FOR PCB TESTS UTILIZING PRELIMINARY ANALYTICAL PROCEDURES

MONS 056519

TABLE 19. BLOOD SAMPLES TESTED BY THE DEGRADATION-DERIVATIZATION TECHNIQUE†

Traverse City Sample ID#	Total PCB as Decachlorobiphenyl in Parts Per Million					
	Sample Date					
	Nov. 1973	Apr. 1974	May 1974	6-25-74	6-26-74	July 1974
00300*	0.015					
00302*		0.020				0.018
00303*	0.025	0.014				0.025
00305*		0.006				0.006
00332**	0.026	0.032	0.023	0.017	0.015	
00333**	0.015	0.008	0.017	0.014	0.005	
00350*	0.028					0.017

Manistee Sample ID#	Total PCB as Decachlorobiphenyl in Parts Per Million		
	Sample Date		
	10-2-74	10-2-74	Nov. 1974
00257	0.075	0.074	0.047
00258	0.060	0.072	0.079
00261	0.032		
00262	0.022	0.030	
00268	0.038	0.036	0.047
00269	0.008	0.034	0.014

† Recovery of decachlorobiphenyl by this technique was 65%

* Single extraction used

** Triple extraction used

*** Accuracy questionable

MONS 056520

A P P E N D I X C

PHYSICAL DATA FOR STUDY PARTICIPANTS

MONS 056521

TABLE 20. PARTICIPANT PHYSICAL DATA - LUDINGTON

I.D.#	Sex	Age	Height	Weight
00200	M	51	71	200
00201	F	44	61	155
00202	F	38	63	159
00203	F	28	66	135
00204	F	58	62	220
00205	M	27	68	158
00206	F	23	64	125
00207	F	64	66	165
00208	F	60	68	192
00209	F	53	63	147
00211	F	48	63	142
00210	M	50	66	150
00212	F	40	64	130
00213	M	38	72	178
00214	M	44	72	160
00215	F	43	62	120
00216	F	41	69	147
00217	M	43	74	208
00218	F	43	61	115
00219	M	50	72	194
00220	F	49	69	150
00221	M	18	73	170
00222	M			
00223	M	26	71	175
00224	M	53	71	210
00225	M	39	70	170
00226	F	36	65	155
00227	M	38	70	160
00228	M	37	67	225
00229	F	33	62	125
00230	F	29	64	102
00231	F	31		
00232	M	33	72	205
00233	M	42	72	213

MONS 056522

TABLE 20. PARTICIPANT PHYSICAL DATA - MANISTEE

I.D.#	Sex	Age	Height	Weight
00250	M	68	62	180
00251	F	38	70	152
00252	M	43	67	143
00253	F	43	65	138
00254	F	59	62	120
00255	F	54	63	148
00256	M	62	62	115
00257	F	53	65	140
00258	M	55	72	180
00259	M	66	73	208
00260	F	64	65	144
00261	M	56	69	200
00262	F	54	67	140
00263	M	51	76	234
00264	F	51	61	114
00265	M	23	75	230
00266	M	62	67	167
00267	F	59	63	123
00268	M	43	69	165
00269	F	36	65	230
00270	M	45	69	160
00271	F	42	64	153
00272	M	45	69	185
00273	F	49	64	180+
00274	M	48	68	155

MONS 056523

TABLE 20. PARTICIPANT PHYSICAL DATA - TRAVERSE CITY

I.D.#	Sex	Age	Height	Weight
00300	F	78	58	140
00301	F	50	66	155
00302	F	63	70	165
00303	F	67	64	142
00304	F	73	60	125
00305	F	41	69	172
00306	M	56	68	175
00307	F	53		
00308	F	64	63	222
00309	M	38	75	220
00310	F	36	69	200
00311	M	53	70	225
00312	F	55	60	130
00313	F	21	57	
00314	F			
00315	M	.58	71	176
00316	F	33	67	130
00317	F	56	67	145
00318	M	26	71	147
00319	M	43	72	165
00320	F	22	65	130
00321	F	56	62	120
00322	F	47	64	145
00323	M	31	69	165
00324	F	31	65	180
00325	F	58	61	140
00326	M	58	68	152
00327	F	52	61	
00328	F	41	62	140
00329	M	57	66	160
00330	F	47	62	120
00331	M	50	67	180
00332	M	58	69	175
00333	F	52	67	135
00334	M	41	69	175
00335	F	41	65	135
00336	M	63	70	170
00337	F	56	65	212
00338	M	57	72	179
00339	F	69	65	139
00340	M	60	70	170
00341	M	60	66	135
00342	M	27	71	160
00343	F	27	68	138
00344	M	23	72	170
00345	F	23	64	125
00346	M	53	65	140
00347	F	51	62	110
00348	M	29	71	160
00349	F	26	66	117
00350	M	42	70	185

MONS 056524

TABLE 20. (continued)

I.D.#	Sex	Age	Height	Weight
00351	F	40	65	135
00352	M	45	69	162
00353	F	44	67	140
00354	F	51	67	130
00355	F	48	66	165
00356	M	47	74	220
00357	F	54	66	180
00358	M	69	67	160
00359	M	36	74	300
00360	F	34	62	160
00361	M	62	68	155
00362	F	66	67	155
00363	M	28	69	170
00364	F	22	65	170
00365	M	64	68	165
00366	F	62	67	158
00367	M	58	67	180
00368	M	43	70	170
00369	F	58	63	125
00370	M	35	70	180
00371	F	35	64	120
00372	F	38	61	118
00373	M	58	70	150
00374	F	21	66	130

TABLE 20. PARTICIPANT PHYSICAL DATA - SOUTH HAVEN

I.D.#	Sex	Age	Height	Weight
00035	M	28	72	165
00036	F	24	65	125
00053	M	23	72	195
00055	M	51	71	164
00062	M	53	73	185
00064	M	44	70	174
00065	M		72	165
00066	F	34	65	115
00067	M	39	72	180
00070	M	57	72	162
00071	F	29	63	120
00073	M	48	67	169
00106	F	36	66	160
00105	M	37	77	210
00114	M	45	72	
00115	F	47	63	110
00120	F	50	68	135
00122	M	46	70	222
00124	M	55	72	126
00126	F	39		
00128	M	62	71	192
00130	F	59	65	160
00116	M	41	71	175
00104	M	35	72	172
00140	F	61	66	140
00139	M	61	68	150
00119	M	45	70	185
00111	M	33	70	200
00121	M	38	70	165
00116	M	41	70	175
00072	F	48	67	140

MONS 056526

TABLE 20. PARTICIPANT PHYSICAL DATA - ALGONAC

I.D.#	Sex	Age	Height	Weight
10013	F	51	68	128
10029	F	59	68	185
11029	M	55	68	161
10033	M	72	70	176
10035	M	66	67	161
10100	M	52	74	220
10110	M	34	71	220
10111	F	31	66	125
10118	M	58	69	220
10129	M	64	65	155
10130	F	50	62	107
10138	M	73	68	193
10150	M	33	72	188
10166	F	48	67	146
10168	F	48	66	242
10169	M	56	66	170
10170	M	71	70	160
10084	M	69	68	150
10085	M	35	67	180

MONS 056527

A P P E N D I X D

SELECTED ILLUSTRATIONS OF THE CONCENTRATION OF PCB IN THE BLOOD OF PARTICIPANTS WHO CONSUMED GREAT LAKES FISH DURING THE STUDY

MONS 056528

APPENDIX D

Illustrations of the correlation between PCB levels in blood and time for persons consuming 26 or more pounds of Great Lakes fish per year. PCB values from Tables 4, 5 & 6.

Legend for Figures 10 and 11.

Participant 00323: Male 31 years, 1974 weight 165 lbs., 1973-75 mean annual fish consumption 64 lbs./yr.

Participant 00328: Male 41 years, 1974 weight 140 lbs., 1973-75 mean annual fish consumption 58 lbs./yr.

Participant 00336: Male 63 years, 1974 weight 170 lbs., 1973-75 mean annual fish consumption 35 lbs./yr.

Participant 00213: Male 38 years, 1974 weight 178 lbs., 1973-75 mean annual fish consumption 132 lbs./yr.

Participant 00324: Female 31 years, 1974 weight 180 lbs., 1973-75 mean annual fish consumption 29 lbs./yr.

Participant 00226: Female 36 years, 1974 weight 155 lbs., 1973-75 mean annual fish consumption 132 lbs./yr.

Participant 00312: Female 55 years, 1974 weight 130 lbs., 1973-75 mean annual fish consumption 49 lbs./yr.

Participant 00363: Male 28 years, 1974 weight 170 lbs., 1973-75 mean annual fish consumption 65 lbs./yr.

Participant 00357: Female 54 years, 1974 weight 180 lbs., 1973-75 mean annual fish consumption 190 lbs./yr.

Participant 00309: Male 38 years, 1974 weight 220 lbs., 1973-75 mean annual fish consumption 42 lbs./yr.

Participant 00337: Female 56 years, 1974 weight 212 lbs., 1973-75 mean annual fish consumption 33 lbs./yr.

Participant 00310: Female 36 years, 1974 weight 200 lbs., 1973-75 mean annual fish consumption 22 lbs./yr.

MONS 056529

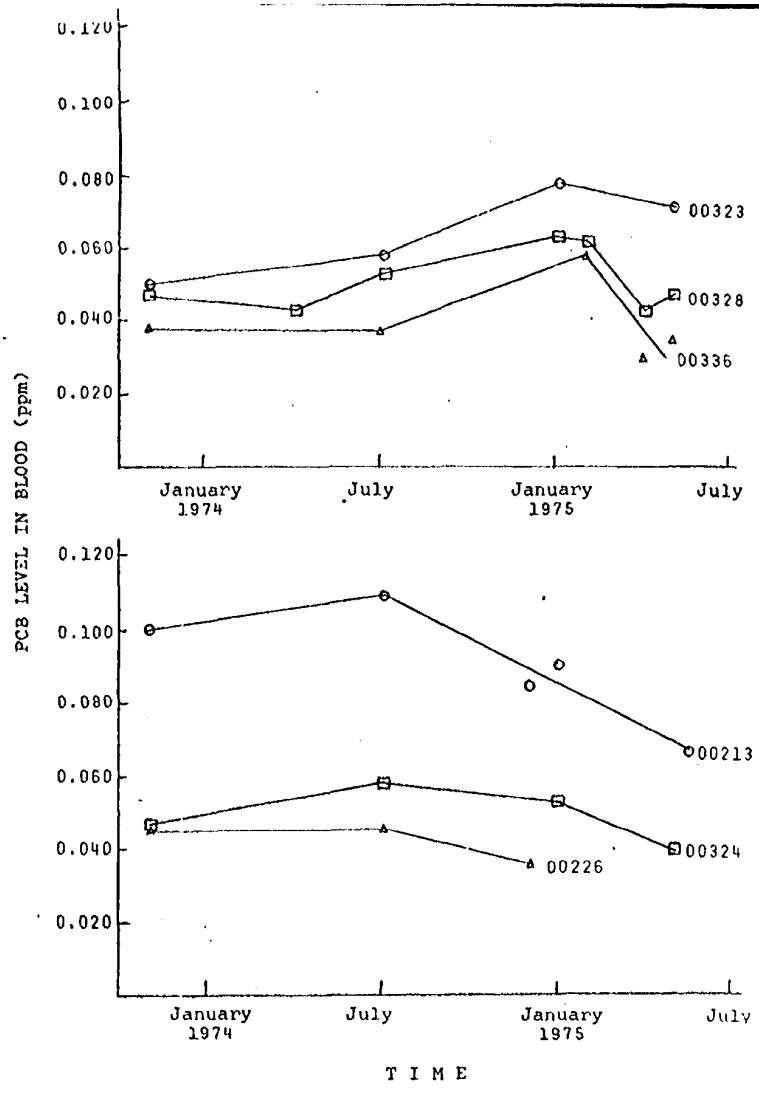

FIGURE 10. CHANGE IN BLOOD PCB LEVELS OVER TIME FOR PERSONS EXPOSED TO LAKE MICHIGAN FISH

MONS 056530

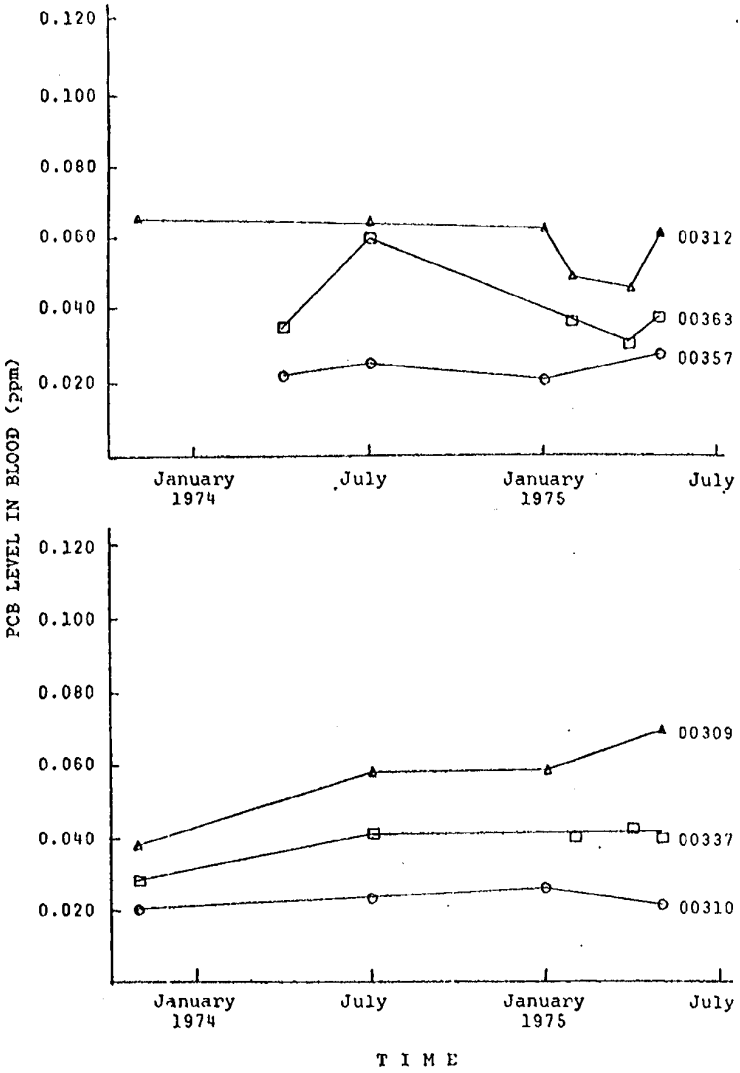

FIGURE 11. CHANGE IN BLOOD PCB LEVELS OVER TIME FOR PERSONS EXPOSED TO LAKE MICHIGAN FISH

MONS 056531

A P P E N D I X E

SELECTED ILLUSTRATIONS OF THE CONCENTRATION OF PCB
IN THE BLOOD OF PARTICIPANTS WHO
ABSTAINED FROM EATING GREAT LAKES FISH DURING 1975

MONS 056532

APPENDIX E

Illustrations of the correlation between PCB levels in blood and time for persons who abstained from consuming Great Lakes fish for various periods of time. PCB values from Tables 4, 5 & 6.

Legend for Figures 12 and 13.

Participant 00338: Male 57 years, 1974 weight 179 lbs., 1973-75 mean annual fish consumption 80 lbs./yr.

Participant 00312: Female 55 years, 1974 weight 130 lbs., 1973-75 mean annual fish consumption 49 lbs./yr.

Participant 00210: Male 50 years, 1974 weight 150 lbs., 1973-75 mean annual fish consumption 24 lbs./yr.

Participant 00330: Female 47 years, 1974 weight 120 lbs., 1973-75 mean annual fish consumption 62 lbs./yr.

Participant 00352: Male 45 years, 1974 weight 162 lbs., 1973-75 mean annual fish consumption 30 lbs./yr.

Participant 00356: Male 47 years, 1974 weight 220 lbs., 1973-75 mean annual fish consumption 100 lbs./yr.

Participant 00355: Female 48 years, 1974 weight 165 lbs., 1973-75 mean annual fish consumption 28 lbs./yr.

Participant 00267: Female 59 years, 1974 weight 123 lbs., 1973-75 mean annual fish consumption 24 lbs./yr.

Participant 00217: Male 43 years, 1974 weight 208 lbs., 1973-75 mean annual fish consumption 24 lbs./yr.

Participant 00218: Female 43 years, 1974 weight 115 lbs., 1973-75 mean annual fish consumption 10 lbs./yr.

MONS 056533

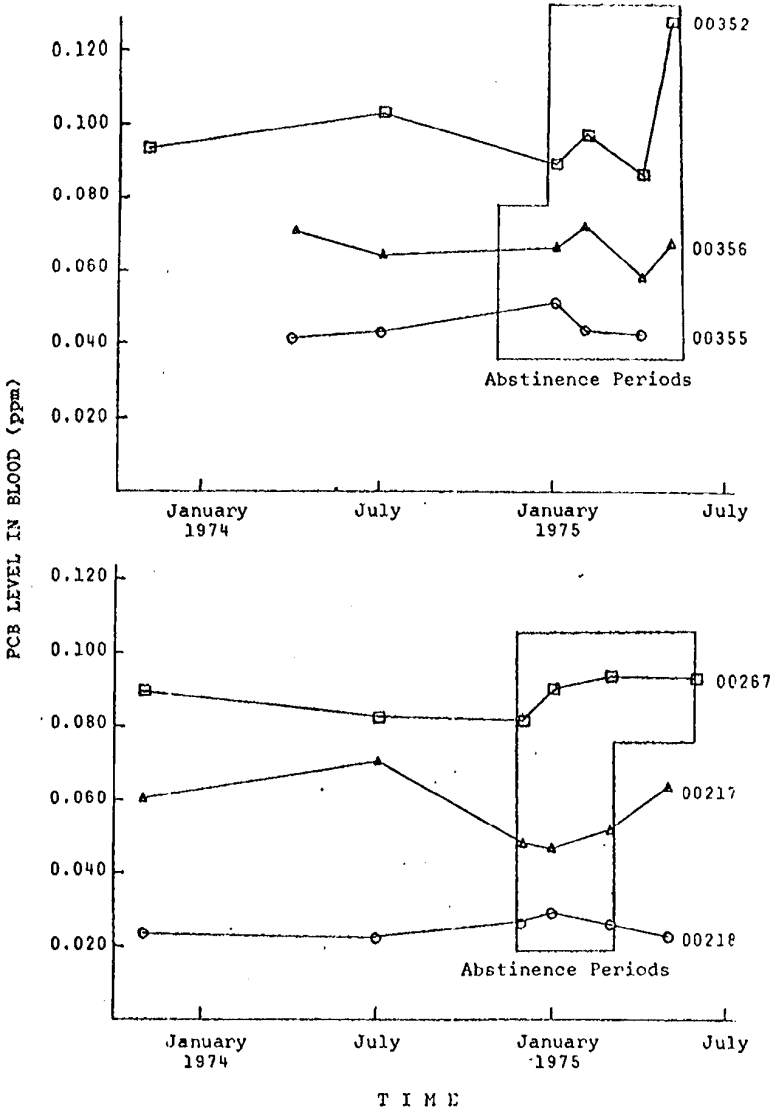

FIGURE 12. CHANGE IN BLOOD PCB LEVELS OVER TIME FOR PERSONS WHO ABSTAINED FROM LAKE MICHIGAN FISH CONSUMPTION

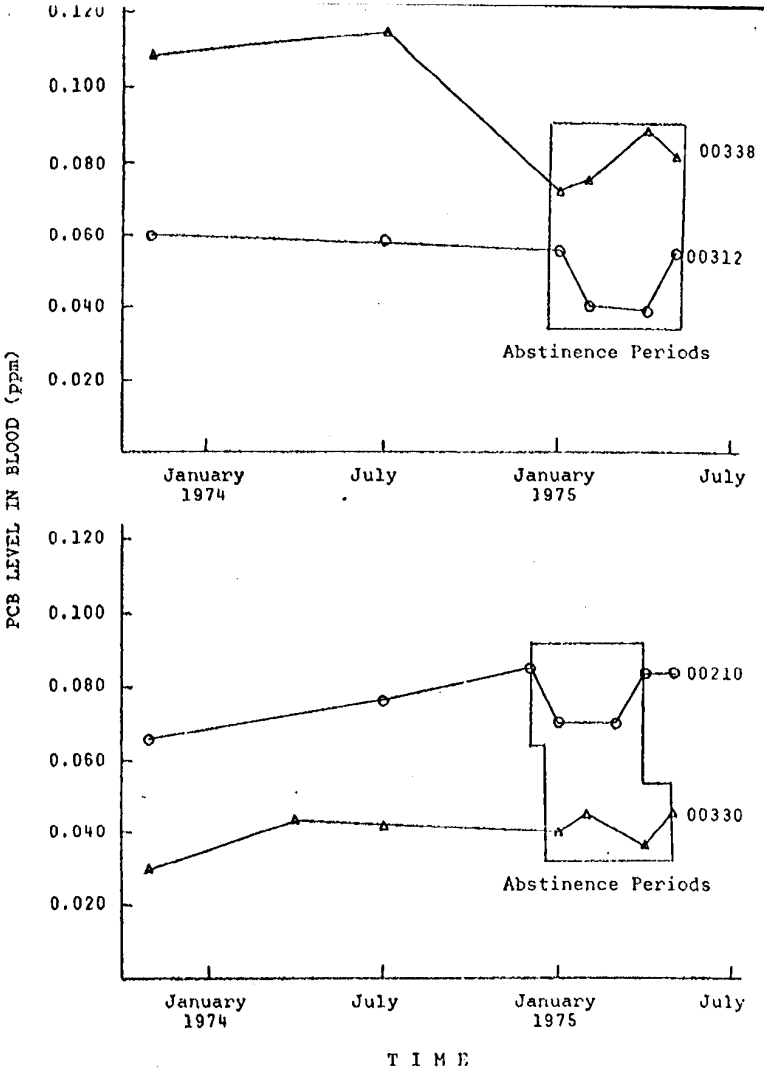

FIGURE 13. CHANGE IN BLOOD PCB LEVELS OVER TIME FOR PERSONS WHO ABSTAINED FROM LAKE MICHIGAN FISH CONSUMPTION

MONS 056535

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In talking with Dr. Kelly before these three paragraphs were written, we agreed that they might as well be phrased so that they could be used not only in the Aroclor booklet, but quoted in correspondence as that may be necessary.

L.A. Watt

Attachment 3-2

3.2

PCB POISONING AND POLLUTION

Edited by
Kentaro HIGUCHI
Fukuoka University Hospital, Japan

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Fig. 7.15 Swelling of the eyelids and brownish skin pigmentation of a new-born baby.

Fig. 7.9 Nail pigmentation (S. K., aged 5, female).

Fig. 7.10 Pigmentation of the palpebral conjunctiva and limbal conjunctiva.

Fig. 7.11 Gingival pigmentation (N. K., aged 36, female).

Fig. 7.12 Pigmentation of the lips.

MONS 056554

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MONS 056555

Preface

Recently, the various health hazards and dangers of indiscriminate discharge of industrial wastes into the environment have been emphasized. Also, strong warnings have been issued, both officially and unofficially, against polluting the environment with toxic or otherwise harmful industrial products. However, it is distressing that, so often, severe cases of poisoning are required before the dangers are fully recognized or effective countermeasures can be implemented or enforced.

The present book covers the broad field of PCB poisoning and pollution in Japan. It emphasized the case of direct human poisoning by oral intake of PCB-contaminated rice-oil (Yusho), and also includes sections on the experimental and toxicological aspects of PCB poisoning, and on the general pollution of the natural environment by PCB's.

The medical description of Yusho covers epidemiologic, pathologic, clinical and therapeutic studies, and there are detailed descriptions of the dermal symptoms (which resemble chloracne) and other major clinical manifestations of the disease, in both adults and children. The sections on toxicology cover various studies, mostly animal experiments, on the physiological and other effects of PCB's, and on the absorption, distribution and metabolism of PCB's in the animal body. The sections devoted to environmental pollution in Japan describe both the microanalytical methodology for PCB's, as adopted in Japan, and also the extent of pollution of the atmosphere, freshwater and marine environments, soil, agricultural products, fish, birds, and man.

As editor for this project, I wish to express my appreciation to all authors for their contributions, and would also like to take this opportunity to thank the staff of Kodansha for their editorial and linguistic assistance in the preparation of the English manuscripts comprising this book.

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MONS 056556

Contents

MONS 056557

MONS 056558