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

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DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

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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 carcinogenesis, of exposure to PCBs and PBBs in various animal models, their relationship to known human effects, and the establishment of dose-response curves.

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4. Human Exposure

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

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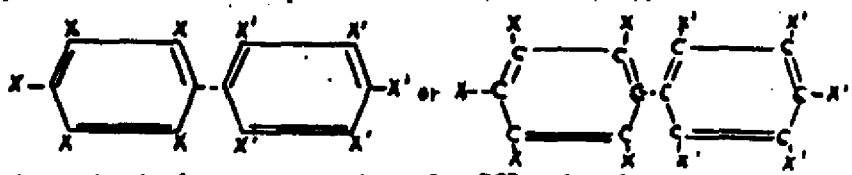
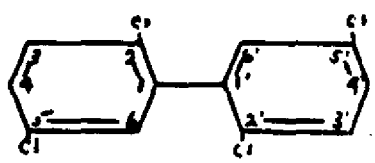
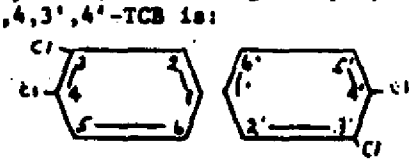
1. Commercial PCBs are complex mixtures. Aroclor 1254, for example, contains 69 different molecules (Zitko, 1971), which differ in the number and position of chlorine atoms.



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:



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 associated 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 conference brought together the latest data, and scientific expertise, to consider all aspects of the verious 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

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.

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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 laborstory 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 chlorinated 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

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,

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 become 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.

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

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

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 PBBs 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 formulations contain trace amounts of chlorinated dibenzofurans (Araki, 1974). These compounds may be up to 170 times more potent as MFO inducers than the PCBs. The chlorinated dibenzofuran concentration of American

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 activetion, 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 laborstory animals have been shown to cause prophyris (Goldstein et al., 1975), disfunction of the thyroid (Bastomsky, 1974), inhibition of verious 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 polar compounds, and that long term PCB storage is in the skin and adipose tissue (Matthews and Anderson, 1975a). Studies of PBB

metabolism are not yet available. Since tissue samples from birds and mammals with known exposures to PCBs usually contain only those PCBs 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

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 elicit 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 affect 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

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

in smooth endoplasmic retiulum, lipid accumulation at higher dietary levels (20 ppm for Aroclor 1254 and 1260) and ultrastructural changes such as atypical mitochondria and the formation of "fingerprints" in the hepatic cytoplasm ("fingerprints" consist of concentrically arranged membranes surrounding lipid vacuoles). An increase in mitotic figures and cell breakdown are increasingly noted with either higher doses or longer exposure (Kimbrough, 1975). 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) and Aroclor 1260. Some tumors have also been produced with Aroclor 1242, 1254 and 1260 (Calandra 1976) in rats in a separate study. In one study with Aroclor 1260, some of the tumors were classified as hepatocellular carcinomas (Kimbrough et al., 1975). The dietary levels of the PCBs in all of these studies were 100 ppm or more, a high level when compared to the daily average human intake of PCBs but not very high for subgroups with a high intake of fish from polluted waterways or in some occupational situations. This is emphasized by the fact that the dietary levels which produce tumors and the relatively low dietary levels which affect reproduction (2.5 ppm in the monkey and 1 ppm in the mink) of some commercial PCB mixtures do not represent a no-effect level. It is presently not known what the no-effect levels are. Other uncertainties

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 effect 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

naphthalenes are toxic within the same dosage range as the chlorinated 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 effect 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 predominantly 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.

HUMAN EXPOSURE

Several reports (Jelinek & Corneliussen, 1975; Humphrey, 1976; Kutz and Yang, 1975; Dennis, 1975; Kleinhert, 1975; Hesse, 1975; Yobs, 1972; Kutz and Strassmman, 1975) provide evidence that would indicate that a substantial proportion of the population of the United States has been exposed to PCBs. Minimal human expoeure of the population to PCBs has occurred from food, air and water, while significant human exposure appears to be limited to aports fishermen consuming fresh water fish from contaminated atreams and lakes, and to occupatibnal 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 parcent of these composites continue to contain detectable residues of PCBs, 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 PCBs 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.

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 PCBs 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

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

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.

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

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 occurred 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 PCBs 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

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.

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.

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

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 PBBs, 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 dibenzofurens.

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 raported 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 characterize 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.

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 bromineted 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

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.

The toxicology of known PCB metebolites should be studied.

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

People who are suspected to heve 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

Inhaletion studies with "heated" PCB mixtures simulating occupational exposure and dermal toxicity studies should be conductad.

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.

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

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.

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.

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

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,

including the newer preperation deeignated ae Aroclor 1016, ie now available (Mieure et al., 1976). Anelysis of the chlorinated biphenyl content of verious American commerciel PCB mixturee (Aroclor) hee been reviewed by Hutzinger et el. (1974). An estimated 40 to 60 different chlorinated biphenyl compounde are present in each of the higher chlorinated commercial mixture. (There are 209 possible compounds obtainable by eubstituting chlorine for hydrogen on from one to ten different poeitions on the biphenyl ring system; see Appendix.) PCB commerciel mixtures produced in the U.S. and eleewhere have been ehown to contein clesses of compounds other then the chlorinated biphenyls: chlorinated napthelenes and chlorinated dibenzofurans (Cl-DBFs), for example (Vos et al., 1970; Roech and Pomerantz, 1974.e,b; Bowes et al., 1975e). The poseibility that nephthalene end dibenzofuren conteminate the technicel biphenyl feedetock used in preparation of the commerciel PCB mixtures hes not been excluded.

Aveilable toxicity information indicates that, of the identified typee of contaminents in commercial PCB mixtures, Cl-DBFs pose the greateet potentisl hazard (See Section II.E on Chlorinated Dibenzofurans). As e result, the Cl-DBFs have become the focus of studies on contaminants in these mixtures. No report has eppeared of an attempted complete content analysis of the trece impurities in e commercial PCB mixture.

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 seperation (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%

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; Societa d'Electro Chimie, 1962). Heating 2,5-dichlorobiphenyl with sodium methoxide produces 2-chloro-5-biphanylol (deCrauw, 1931). Decachlorobiphenyl when treated with sodium methoxide in pyridine yields the 4-methoxy- and 4,4'-dimethoxy-chlorobiphenyl (Binns 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

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 sensitors 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.

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 prefarentially. 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'-hexachlorobiphanyl 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

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 207913

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 are data (Mieure et al., 1975) which indicate 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 are 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 -207914

The relationships between structure and bioaccumulation factors for PCB isomers are only beginning to be assessed (McKinney, 1975; Sugiura at 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 more 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 water 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

clearly underatood, 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 at al., in press) and subsequently in mice (Biocca, 1975) has demonstrated that seperate 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 tha role of metabolism here, the data would suggest a

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

received little attention for obvious reasons. Recently, the metabolism of purified chlorinated biphenyls, 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.

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 problem 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 Dispoaition, 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

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. Analysis 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),

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 chlorobiphanyls 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 Analytical 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

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.

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 pesticidas 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 Peet, 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

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 interpretetions 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 anslysis.

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 then 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

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 seperated; 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

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 quantitetively to the dichlorobensophanone 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

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; Beazhoold 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 countarpart 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; Beazhold 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

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.

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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 addad 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 Exploratoin 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 ±

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

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environmental samples (Trottar, 1975; Jensen at al., 1972; Giam and Wong, 1972). Parchlorination 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 (Trottar 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; Rosch and Pomerantz, 1974; Bowes, et al., 1975; Porter and Burke, 1971). The chlorinated dibenzofurans have gas chromatographic properties similar to the PCBs (Bowes, 1975).

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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 seperation 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

about 0.2 ppm for individual foods and about 0.05 ppm for Total Diet composites. Fish from certain fresh water locetions 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 e greater potential danger for several reasons. In using a chick bioassay to monitor fractionation of commercial PCB mixtures, 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).

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. 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 (Curlay at 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).

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

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 evidance 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. Interpretation of results from the testing of individual chlorinated biphenyl compounds, however, may not be entirely straight forward. In the Ullmann coupling reaction of

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 el., 1973). This hexechlorobiphenyl is a significant constituent of PCB mixtures (Sissons and Welti, 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

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-conteining heet 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

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 relative 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

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

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 (Kerst. 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

throughout most of the world. Many of these locetions may offer conducive settings for chemicel end metebolic conversions. PCB residues in e 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 stebility of PBBs to oxidation has not been studied and documented in the literature. FireMaster BP-6 is unstable to elkaline hydrolysis. After refluxing FireMaster BP-6 with 2% KOH in ethenol, GLC chromatograms show erretic degradation of hexabromobiphenyl (DET,DO,DCT,DCH,1976), the major component of FireMaster BP-6. The possible rete of PBB hydrolysis in the environment under milder conditions is not known.

Photochemistry

Recent anelysis of FireMaster BP-6 by GCMS using an OV 101 column demonstrated 12 peeks whose mass spectrum corresponds to 2 pentas, 4 hexas, 4 heptas and 2 octas. However, about 50 percent of the meterial 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).

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. enhenced intersystem crossing to a triplet state due to vibronic coupling with the bromines, 2. steric interferance 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

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

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 then 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 effected 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).

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.

METABOLISM AND BIOCHEMICAL TOXICITY OF PCBs and PBBs

I. Effects of PCBs on Biochemical Functions

One of the major biochemical affects of PCBs is the induction of microsomal enzymes in the liver. Risebrough et al., (1968) suggested that PCBs have the capability to induce the activities of microsomal enzymes. Subsequently, Street et al., (1969) demonstrated the induction of liver enzymes in rats by PCBs. 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 (Villanauve 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 PCBs vary between 0.5-25 ppm. (Litterst et al., 1972b; Villensuva et al., 1971; Turner et al., 1974).

The time-course of microsomal syzyme 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

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in the diet. A single oral dose in rats resulted in maximum enzyme activities at 24 hours. Bickets at 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 Ivarson 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, Chen et al., 91973b) found that both

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 benzopyrene 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 <u>in vitro</u>. Several workers indeed have observed enzyme induction effects <u>in vivo</u> by demonstrating shortened barbiturate sleeping times (Bickers et al., 1972; Johnstone et al., 1974: Villeneuve et al., 1972):

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.

Alvaras & Kappas (1975) reported the induction of hydroxylase and demethylase in placenta, as well as in the fetus, when pregnent 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. Benzopyrene 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 ultraviolet 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

idea was supported by Grots et al., (1975). Aroclor 1242 was found to be a more potent inducer of liver porphyris than Aroclor 1016 (Goldetein 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 protain rther than to the enzyme induction. Wit (1972) has shown that PBBs which are similar to PCBs are potent hepto-porphyrinogenic 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

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 Wiss, (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 al., 1971; Cutkomp et al., 1972; Desaieh 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).

Sivalingam 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.,

(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 phosphatidychlolins 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 conteining 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

Fumiko (1972) in rats and by Orberg, and Kihlstrom (197) in mice. Orberg et al., (1972) found that the estrus cycle of the mouse increased in length after a single injection of PCBs. Linder 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 dehydrospiandrosterone 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.

These changes were concomitant 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 concantrations 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 diatary PCBs increased the incidance 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

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

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

A preliminery 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 giva an estimate of the relative biological activity of these chemicals in many 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

considered tentative until similar 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.

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 with 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

than in those fed Aro and is consistant 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 affects of Aro and FM were often elimineted.

A summary of the major affects 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 anzyme 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 environmentel 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

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

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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.

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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

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TABLE 3 (Cont'd)

	LEL[1] ppm		Maximum Effect % Control	
	Aro	FM	Aro	FM
PARAMETERS				
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

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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.

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chlorinated materials in the environment, the observation has led to the general belief that the less chlorinatad 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 readily 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.

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). Coneietant 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 etate" 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'e diet are greatest with PCBs of 54% chlorination. The residus levels in milk are approximately 4 times the reeidue level in the cow's diet. In hens, reeidue 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 tieeues 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 ths animals are fed environmentally realistic levels.

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.

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

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

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 Andersen, 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

to result in dechlorination and/or shifting of a chlorine atom (Daly et al., 1972). The metabolism of selected hexachlorobiphenyls has been reported (Jansen 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.

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 extensively the toxic effects produced in fish by PCBs, 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,

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 associated 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 staxis, 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.

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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.

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	
" 1240	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'-tatrachlorobiphenyl	Mice(CFI strain)	intraperitoneal	2.15	Yamamoto & Yoshimura (1973)
5-OH derivative of 2,4,3'4'-tatrachlorobiphenyl	Mice(CFI strain)	"	0.43	"
2,3,4,3'4'-pentachlorobiphenyl	Mice(CFI strain)	"	0.65	"

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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

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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	

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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) for 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	"

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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).

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).

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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 at 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).

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 exhibitad only moderate periorbital edema and arythema. 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).

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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-

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

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 only resulted 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 (Keplinger, 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,

2,3,6,2',3',6', hexachlorobiphenyl and 2,4,5,2',4',5' hexachlorobiphenyl for 21 days at a laval of 400 ppm. The isomer 2,4,5,2',4',5' hexachlorobiphenyl was also fad 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 viaceral 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 Kanechlor 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 ± 2.5 days to 8.7 ± 4.3 days (Orberg and Kihlstrom, 1973; Kihlstrom et al., 1973). Females treated analogously for 62 days

end 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 significantly 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 ware 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 were 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

at 100 ppm. No reproductive affects 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.

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 (evidanced 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 matings, 12.5% of the 5 ppm group and 37.5% of the 2.5 ppm group was pregnant

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 (Aftosmis 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

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, 100, 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 (Aftosmis et al., 1972a).

When 1 g/kg of octobromobiphenyl was applied in corn oil under occluded 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

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

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 enorexic, would consume only milk and developed signs of hyperkeratosis over the antire 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 significantly 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).

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 reaponse 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 e 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-tetrachlorodibenzofuren 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

ear caused severe hyperkeratosis at the application site.

The single oral LD_{50} for tetrechlorodibenzofuran 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 thymus but no fatalities (Moore et al., 1976).

While these findings indicate that in mice TCDF is 30 fold less toxic then 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.

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,

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, B, or Y 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 B BHC. Thus PCBs enhanced the tumor development of liver tumors of α or B 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

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; Buree, 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).

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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 matatases 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 cercinogens in Sprague Dawley rats such as 3'mathyl-4-dimethylamino-azobenzene, N-2-fluorenyl acetamide and diethylnitrosamine was inhibited by Kanechlor 500 (Makiure 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

onset of the experiment, 5 rats per each group and sex were sacrificed. The surviving rats were sacrificied 24 months after onset of the experimant. 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 hyperpleaia, 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

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 peritonitis 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.

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 auppress 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 conteminants 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 buraal follicles and in the spleen. It suppressed the humeral immune response in guines 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 mutegenic 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

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 demonstrated e decrease in the frequency of implanted ova

among young animals nursed on milk conteining PCB (Clophen 60). Both the male end 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 end Chen, 1972; Berlin et el., 1975; Malvas and Brandt, 1973; Brandt and Ullberg, 1973). Evidence of placantal transfer was also observed among Yusho patients. While PCB has no known or clearly defined teratogenic effect in mammels, 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

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. 'Praliminary Environmental Hazerd Assessment of Chlorinated Naphthalenes, Silicones, Fluorocarbons, Benzenepolycarboxylates and Chlorophenols'. (NTIS accession number PB-238-074/AS)

(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-	House	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.

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(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 hyperkaratosis.
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

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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.

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, lecrimation, 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

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 cirrhosis of the liver and cardiovascular effects.

Toxicity in Man

The most important route for man's 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 often concurrent syndromes have been described, namely, liver necrosis, and chloracne

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. Does response effects have not been established.

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

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 apparantly persist, as reflected by results obtained in continued food sampling.

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 aquatic environment.

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

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 ratee 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 date 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 tamporary 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 raported 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

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 the 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.

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

TABLE 10

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
Ha[illegible]ck*	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

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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

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 12.

Table 11 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 10 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.

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TABLE 11

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 crabs 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.

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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 <u>older larger oily fish</u>, especially those that spend part or all of their lives in contaminated lakes, rivers or estuaries.

Table 12 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

TABLE 12

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
[illegible]rican 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.

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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 13 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 13 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 13, an estimate can be made that about 200,000 U.S. consumers would be exceeding the 200 mcg/day FDA limit. As

TABLE 13

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.

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 200 mcg/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.

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

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 tamporary tolerences. 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 tha 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

that many surface water samples contained PCBs at concentrations above the detection limit of 10 ppt. Ten 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

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 raported quantity of Lake Michigan fish consumed and the concentration of PCB in the blood of study participants. Using a natural log transformation

of PCB values, the correlation between amount of fish consumed and PCB blood levels was significant (t=6.24, p= 0.0001), with higher raported 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 avereged 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. Becsuse fish consumption was found to vary from year to year the averege annual consumption for each individual for the two baseline years of study was used in each case.

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 greater 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.

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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 mg. In this same study, the lowest dose leading to overt symptomatology was 500 mg.

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 per

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."

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 GLC/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

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 diminution 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 (?)

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 Oeme, 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

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 Nagayama 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; Kuratsune 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.

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 et al., 1975a), than 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 ralated 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.

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), (Drinker et al., 1937), (Good et al., 1943), (Schwartz, 1943) and (Maigs, et al., 1954) of cutaneous eruptions and of systemic manifestations (sometimes fetal) 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.

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 biphanyls 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 no 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

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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, Karppshen 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.

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 14 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 tha "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 voluntear 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).

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)

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years) having no history of occupational exposure to PCB's. Study parameters 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 effects 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 15 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 16 shows that there was no lowering of blood PCB concentration among those workers tested two months after the installation of a more

TABLE 15

Aroclore 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)

TABLE 16

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)

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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

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, respectivaly, 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.

Although no measurements were available, Hasegawa et al., (1973) estimated that environmental conditions in carbonless copy paper manufacturing facilities (using Kanachlor 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 and hepatic "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-?)

examination. A 1973 notice advised 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 dermatologic 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.

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 PCBs 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 PCBs handled in the factory, and that, conversely, the PCBs found in the workers' blood contained one more chlorine atom than did the PCBs handled in the factory, i.e., if the PCBs 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.

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.

Segami 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 subcuteneous 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

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

of the State.

Haase (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) has 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

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 prevalant in Aroclor 1254 and Aroclor 1260. Additional analysis indicated that the most frequently encountered PCB residues were penta-, haxa-, and haptachlorobiphenyl 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 Canadiens 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

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.

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

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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 degredation 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.

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

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 probably 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

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 17).

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 18).

Table 17

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 18

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 19).

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TABLE 19

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.

BIBLIOGRAPHY

AD HOC COMMITTEE OF JAPAN PUBLIC HEALTH ASSOCIATION. (1974), Environmental Health Criteria for Polychlorinated Bi- and Terphenyls. Environmental Agency of Japan/WHO Environmental Health Criteria Programme.

Aftosmis, J. G., Culik, R., Lee, K. P. (1972), Toxicology of Brominated Biphenyls: I. Oral Toxicity and Embryotoxicity, Toxicol Appl Pharmacol 22:316.

Aftosmis, J. G., Culik, R., Lee, K. P., Sherman, H., and Waritz, R. S. (1972a), The Toxicology of Brominated Biphenyls: I. Oral Toxicity and Embryotoxicity, Presented at the Society of Toxicology Meeting in Williamsburg, VA, Toxicol Appl Pharmacol 22:316.

Aftosmis, J. G., Dashiell, O. C., Griffith, F. D., Hornberger, C. S., McDonnell, M. E., Sherman, H., Tayfun, F. O., and Waritz, R. S. (1972b), Toxicology of Brominated Biphenyls: II. Skin, Eye, and Inhalation Toxicity and an Acute Test for Evaluating Hepatotoxicity and Accumulation in Body Fat, Toxicol Appl Pharmacol 22:316-317.

Albro, P. W. and Fishbein, L. (1972), Intestinal Absorption of Polychlorinated Biphenyls in Rats, Bull Envir Contam Toxicol 8:26-31.

Allen, J. R. and Norback, D. H. (1973), Polychlorinated Biphenyl and Triphenyl Induced Gastric Mucosal Hyperplasia in Primates, Science 179:498-499.

Allen, J. R., Carstens, L. A., and Barsotti, D. A. (1974), Residual Effects of Short-term, Low-level Exposure of Non-human Primates to Polychlorinated Biphenyls, Toxicol Appl Pharmacol 30:440-451.

Allen, J. R., Norback, D. H., and Hsu, I. C. (1974), Tissue Modifications in Monkeys as Related to Absorption, Distribution, and Excretion of Polychlorinated Biphenyls, Arch Environ Contam Toxicol 2:86-95.

Allen, J. R. (1975), Response of the Nonhuman Primate to Polychlorinated Biphenyl Exposure, Fed Proc 34:1675-1679.

Allen, J. R., Carstens, L. A., Abrahamson, L. J., and Marlar, R. J. (1975), Responses of Rats and Nonhuman Primates to 2,5,2',5'-Tetrachlorobiphenyl, Env Res 9:265-273.

Alvares, A. P., Bickers, D. R., and Kappas, A. (1973), Polychlorinated Biphenyls. New Type of Inducer of Cytochrome P 448 in the Liver, Proc Nat Acad Sci 70:1321-1325.

Alvares, A. P., and Kappas, A. (1975), Induction of Aryl Hydrocarbon Hydroxylase by Polychlorinated Biphenyls in the Retroplacental Unit and Neonatal Livers During Lactation, FEBS Lett 50:172-174.

Andersson, K., Norstrom, A., Rappe, C., Raamuson, B., and Swahlen, H. (1974), Photochemical Degradation of PCB, PBB and Other Flame Retardants, 34th International Congress of Pesticide Chemistry, Helsinki, Finland.

Araki, Y. (1974), Comparison of Hepatic Enzyme-Inducing Activities of Chlorinated Dibenzofuran and Chlorinated Dibenzodioxin, Fukuoka-Igaku-Zasshi 65:61-64.

Armour, J. A. and Burke, J. A. (1970), Method for Separating Polychlorinated Biphenyls from DDT and its Analogs, JAOAC 53:761-768.

Armour, J. A. (1972), Gas Chromatographic Data for Polychlorinated Biphenyl Components in Six Aroclors, J Chromatogr 72:275-282.

Armour, J. A. (1973), Quantitative Perchlorination of Polychlorinated Biphenyls as a Method for Confirmatory Residue Measurement and Identification, JAOAC 56:987-993.

Armour, J. A. and Burke, J. A. (1971), Behavior of Chlorinated Naphthalenes in Analytical Methods for Organochlorine Pesticides and Polychlorinated Biphenyls, JAOAC 54:175-177.

Aulerich, R. J., Ringer, R. K., and Iwamoto, S. (1973), Reproductive Failure and Mortality in Mink Fed on Great Lakes Fish, J Reprod Fert Suppl 19:365-376.

AUSTRALIAN NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL. (1970), Atmospheric Contaminents NHJMRC, Canberra.

Ax, R. L. and Hansen, L. G. (1975), Effects of Purified PCB Analogs on Chicken Reproduction, Poultry Science 54(3):895-900.

Ax, R. L., Millar, W. L., and Hansen, L. G. (1976), Toxicity Comparisons of Two Pentachlorobiphenyls. Abstract for presentation at American Society of Animal Science - Midwestern Section.

Bacha, C. A. and Lisk, D. J. (1973), A Qualitative Method for Detecting Hydroxylated Metabolites of Polychlorinated Biphenyls, Bull Environ Contam Toxicol 9:315-317.

Bailey, S., and Bunyan, P. J. (1972), Interpretation of Persistence and Effects of Polychlorinated Biphenyls in Birds, Nature 236:34-36.

Barsotti, D. A., and Allan, J. R. (1975), Effects of Polychlorinated Biphenyls on Reproduction in the Primate, Fed Proc 34:338.

Bastomsky, C. H. (1974), Effects of a Polychlorinated Biphenyl Mixture (Aroclor 1254) and DDT on Biliary Thyroxin Excretion in Rats, Endocrinol 95:1150-1155.

Bastomsky, C. H., Solymoss, B., Zsigmond, G., and Wyse, J. M. (1975), Mechanism of Polychlorinated Biphenyl-Induced Hypobilirubinaemia, Clin Chim Acta 61:171-174.

Bastomsky, C. H., and Wyse, J. M. (1975), Enhanced Thyroxine Metabolism Following Cutaneous Application of Microscope Immersion Oil, Res Commun Chem Pathol Pharmacol 10:725-733.

Bauer, H., Schulz, K. H., and Spiegelberg, U. (1961), Berufliche Vergiftungen bei der Herstellung von Chlorophenol Verbindungen, Arch Gewerbepath Gewerbehyg 18:538-555.

Bauer, H., Schulz, K. H., and Spiegelberg, U. (1961), Occupational Intoxications in Manufacturing Chlorophenol Compounds, Arch Gewerbepath Gewerbehyg 18:538-555.

Baxter, R. A., Gilbert, P. E., Lidgett, R. A., Mainprize, J. H., and Vodden, H. A. (1975), The Degradation of PCBs by Microorganisms, Sci of the Total Environ 4:53-61.

Bazzhold, F. L. and Stout, V. F. (1973), The Use and Effect of Mixed Standards on the Quantitation of Polychlorinated Biphenyls, Bull Environ Contam and Toxicol 10:10-15.

Berg, O. W., Dioxady, P. L., and Rees, G. A. V. (1971), Column Chromatographic Separation of Polychlorinated Biphenyls from Chlorinated Hydrocarbon Pesticides and Subsequent Gas Chromatographic Quantitation in Terms of Derivatives, Report of Ontario Water Resources Commission.

Berlin, M., Gage, J., and Holm, S. (1975), The Distribution and Metabolism of 2,4,5,2',5'-Pentachlorobiphenyl, Arch Environ Health 30:141-147.

Bickers, D. R., Eisaman, J.. Kappas, A., and Alvares, A. P. (1975), Microscopa Immersion Oils. Effects of Skin Application on Gutaneous and Hapatic Drug Metabolizing Enzymas, Biochem Pharmacol 24:779-783.

Bickers, D. R., Harbar, L. C., Kappas, A., and Alvaras, A. P. (1972), Polychlorinated biphenyls. Comparative Effects of High and Low Chlorine Containing Aroclors on Hapatic Mixed Function Oxidaas, Res Comm Chem Pathol Pharm 3:505-512.

Bickars, D. R., Kappas, A., and Alvares, A. P. (1974), Differances in Inducibility of Gutaneous and Hapatic Drug Metabolizing Enzymes and Cytochroma P 450 by Polychlorinatad Biphanyls and 1,1,1-Trichloro-2,-bis (p-Chlorophenyl) Ethane (DDT), J Pharmacol Exp Ther 188:300-309.

Binna, F. and Suschitzky, H. (1971), Polyhaloganoaromatic Compounds. Part XX. Some Reactiona of Decachlorobiphenyl, J Chem Soc (C) 1913-1917.

Biocca, M. (1975), Toxicology of Selactad Symmetrical Hexachloro-biphanyls. Biological Rasponaas in Chicksns and Mics, National Confarence on Polychlorinated Biphenyls, Chicago.

Biocca, M., Moora, J. A., Gupta, B. N., McKinney, J. D. (1975), Toxicology of Salactad Symmetrical Hexachlorobiphenyl Isomers. Biological Rasponses in Chicks and Mica, Nat Conf on Poly-chlorinatad Biphenyls, Chicago.

Bitman, J., and Cecil, H. C. (1970), Estrogenic Activity of DDT Analoga and Polychlorinatad Biphanyls, J Agric Food Chem 18:1108-1112.

Block, W. D. and Cornish, H. (1959), Metabolism of Biphenyl and Chlorobiphenyl in tha Rabbit, J Biol Chem 234:3301-3302.

Bowas, G. W., Mulvihill, M. J., Simonait, B. R. T., Burlingame, A. L., and Riaabrough, R. W. (1975a), Identification of Chlorinated Dibenzofurans in Amarican Polychlorinated Biphenyls, Nature 256:305-307.

Bowas, G. W., Mulvihill, M. J., DeCamp, M. R., and Kends, A. S. (1975b), Gas Chromatographic Characteristics of Authentic Chlorinated Dibenzofurans; Identification of Two Isomers in American Japanese Polychlorinatad Biphenyls, J Agr Food Chem 23:1222-1223.

Bowas, G. W., et al. (1975), Identification of Chlorinatad Dibenzo-furans in Amarican Polychlorinated Biphanyls, Nature 256:305-307.

Brandt, I. and Ullberg, S. (1973), Distribution of Hexa- and Octachloro Biphanyl in Mice and Quails, Report to the National Swedish Environment Protection Board.

Bruckner, J. V., Knanna, K. L., and Cornish, H. H. (1973), Biological Responses of the Rat to Polychlorinated Biphenyls. Toxicol Appl Pharmacol 24:434-438.

Bruckner, J. V., Khanna, K. L., and Cornish, H. H. (1974), Effect of Prolonged Ingestion of Polychlorinated Biphenyls on the Rat, Food Cosmet Toxicol 12:323-330.

Buras, V. W., Kimbrough, R. D., Villanueva, E. C., Jennings, R. W., Lindar, R. E., Sovocool, G. W. (1974), Polychlorinated Biphanyls, Storage, Distribution, Excretion, and Recovery: Liver Morphology After Prolonged Dietary Ingestion. Arch Environ Health 29:301-307.

Burke, J. A. (1972), Report on Chlorinated Insecticides, JAOAC 55:284-287.

Bush, B., Tumasonis, C. F., Baker, F. D. (1974), Toxicity and Persistence of PCB Homologs and Isomers in the Avian System, Arch Environ Contam and Toxicol 2(3):195-212.

Byrne, J. J., Reinake, P., Ringer, R. K., and Aulerich, R. (1975), Influences of Polychlorinated Biphenyl Mixture Aroclor 1254 Administration on Reproduction and Thyroid Function in Mink Mustela-vison, Fed Proc 34:321.

Calandra, J. C.: Summary of Toxicological Studies on Commercial PCBs. Two-year Chronicoral Toxicity Study of Aroclor 1242, 1254, 1260 in beagle dogs. Monsanto Company, St. Louis, MO. 63116.

Cecil, H. C., Harris, S. J., Bitman, J. and Fries, G. F. (1973), Polychlorinated Biphenyl-Induced Decrease in Liver Vitamin A in Japanese Quail and Rats, Bull Environ Contam Toxicol 9:179-185.

Chan, P. R., Mahendale, H. M., and Fishbein, L. (1973b), Effect of Two Isomeric Tetrachlorobiphenyls on Rats and Their Hepatic Enzymes, Arch Environ Contam Toxicol 1:36-47.

Chan, P. R., McKinney, J. D., and Matthews, H. B. (1975), Metabolism of 2,4,5,2'5'-Pentachlorobiphenyl in the Rat: Qualitative and Quantitative Aspects, in preparation.

Chen, T. S., and Dubois, K. P. (1973a), Enzyme Inducing Effect of Polychlorinated Biphenyls, Toxicol Appl Pharmacol 26:504-512.

Cheaney, C. F., and Allen, J. R. (1974), Oxidative Phosphorylation and Respiration by Liver Mitochondria from Polychlorinated Biphenyl Intoxicated Rats, Biochem Pharmacol 23:1577-1582.

Collins, G. B., Holmes, D. C., and Jackson, F. J. (1972), The Estimation of Chlorobiphenyls, J Chromatogr 71:443-449.

Comba, G. F., Jr., Cantor, A. H., and Scott, M. L. (1975a), Effects of Dietary Polychlorinated Biphenyls on Vitamin E and Selenium Nutrition in the Chick, Poult Sci 54:1143-1152.

Cook, J. W. (1972), Some Chemical Aspects of Polychlorinated Biphenyls (PCBs), Environ Health Perspec 1:3-13.

Corbett, T. H., Beaudoin, A. R., Cornell, R. G., Anver, M. R., Schumacher, R., Endrea, J. and Szwabowska, M. (1975), A Toxicity of Polybrominated Biphenyls (Firemaster BP-6) in Rodents, Environ Research 10:390.

Crosby, D. (1969), Experimental Approaches to Pesticide Photodecomposition, Residue Rev 25:1-12.

Crosby, D. B. and Moilanen, K. W. (1973), Photodecomposition of Chlorinated Biphenyls and Dibenzofurans, Bull Envir Contam and Tox 10:372-377.

Crow, K. D. (1970), Chloracne, Trans St Johns Hosp Dermat Soc 56:79-99.

Curley, A., Burse, V. W., Jennings, R. W., Villanueva, E. C., and Kimbrough, R. D. (1975), Evidence of Tetrachlorodibenzofuran (TCDF) in Aroclor 1254^{R} and the Urine of Rats Following Dietary Exposure to Aroclor^{R}, Bull Envir Contam and Tox 14:153-158.

Curley, A., Burse, V. W., Grim, N. E., Jennings, R. W., and Linder, R. E., (1971), Polychlorinated Biphenyls: Distribution and Storage in Body Fluids and Tissues of Sherman Rats, Environ Res 4:481-495.

Cutkomp, L. K., Yap, H. H., Desaiah, H. H., and Koch, R. B. (1972), The Sensitivity of ATP-ases to Polychlorinated Biphenyls, Environ Health Perspect 1:165-168.

Daly, J. W., Jerina, D. M., and Witkop, B. (1972), Arene Oxides and the NIH Shift: The Metabolism, Toxicity, and Carcinogenicity of Aromatic Compounds, Experients 28:1129-1149.

Davis, P. W., Friedhoff, J. M., and Wadameyar, G. A. (1972), Organochlorine Insecticida, Herbicida, and Polychlorinated Biphenyl (PCB) Inhibition of $Na^{+}K^{+}$ -ATPasa in Rainbow Trout, Bull Environ Contam Toxicol 8:69-72.

deCrauw, R. (1931), The Principle of Inducad Alternating Polarity in Connection with the Reactions of Derivatives of p-Dichlorobenzene and Other Compounds with Sodium Methylate, Rec Trav Chem 50, 753-792.

deFreitas, A. S. and Norstrom, R. J. (1974), Turnover and Matabolism of PCBs in Ralation to thair Chemical Structure and the Movement of Lipids in the Pigeon, Can J Physiol 52:1080-1094.

Demming, D. V. M. (1975), Summary of Team Evaluation Effort in Harda Quarantined for Polybrominated Biphenyls (PBB) in a Report from the State Senate Special Investigating Committee (Senator John A. Welborn, Chairman). The Contamination Crisis in Michigan, July.

Dennis, D. S. (1975), Polychlorinated Biphenyls in the Surface Waters and Bottom Sediments of the Major Drainage Basins of the United States, Nat Conference on Polychlorinated Biphenyls, Chicago.

Desaiah, D., Cutkomp, L. K., Yap, H. H., and Koch, R. B. (1972), Inhibition of Oligomycin-sensitive and Insensitive Magnesium Adenosine Triphosphatase Activity in Fish by Polychlorinated Biphenyls, Biochem Pharmacol 21:857-865.

DET-DO, DCT, DCH (Feb. 9, 1976), Polybrominated Biphenyl Methodology, Food and Drug Administration Laboratory Information Bulletin 1705D.

deVos, R. H. and Peat, E. W. (1971), Thin Layer Chromatography Polychlorinated Biphenyls, Bull Environ Contam Toxicol 6:164-170.

Dikshith, T. S. S., Rockwood, W., Abraham, R., and Coulston, F. (1975), Effects of a Polychlorinated Biphenyl (Aroclor 1254) on Rat Testis, Exp Mol Pathol 22:376-385.

Drinker, C. K., Warren, M. F., and Bennett, G. A. (1937), The Problem of Possible Systemic Effects from Certain Chlorinated Hydrocarbons, J Ind Hyg Toxicol 19(7):282-311.

Ecobichon, D. J., and Comeau, A. M. (1975), Isomerically Pure Chlorobiphenyl Congeners and Hepatic Function in the Rat: Influence of Position and Degree of Chlorination, Toxicol Appl Pharmacol 33:94-105.

Ecobichon, D. J., and Mackenzie, D. O., (1974a), Uterotropic Activity of Commercial and Isomerically Pure Chlorobiphenyls in the Rat, Res Commun Chem Pathol Pharmacol 9, 85-95.

Edwards, J. E. and White, J. (1951), Pathology Changes with Special Reference to Pigmentation and Classification of Hepatic Tumors in Rats Fed P-dimethylamine Acobenzene (butter yellow), J Natl Cancer Institute 2:157-183.

Edwards, R. (1974), Some Factors in the Separation of Polychlorobiphenyls (PCBs) from Organochlorine Pesticides by Column Chromatography Combined with Gas-Liquid Chromatography, Pestic Sci 5:293-304.

Erney, R. D. (1975), Confirmation of Polybrominated Biphenyl Residues in Animal Feeds and Dairy Products Using an Ultraviolet Irradiation Gas-Liquid Chromatographic Technique, JAOAC 58:1202-1205.

Farber, T. M. and Bakar, A. (1974), Microsomal Enzyme Induction by Hexabromobiphenyl, Soc Toxicol Meeting, Washington, DC, March 10-14.

Fehringer, N. V. and Westfall, J. E. (1971), Separation and Identification of DDT Analogs in the Presence of Polychlorinated Biphenyl Compounds by Two-Dimensional Thin Layer Chromatography, J Chromatogr 57:397-405.

Fehringer, N. V. (1974), TLC of Polybrominated Biphenyls in Animal Feeds and Dairy Products, Food and Drug Administration Laboratory Information Bulletin, No. 1705.

Fehringer, N. V. (1975), Determination of Polybrominated Biphenyl Residues in Dry Animal Feeds, JAOAC 58:1206-1210.

Ficsor, G. and Wertz, G. F. (1976), Polybrominated Biphenyl Nonteratogenic C-mitosis Synergist in the Rat, Prea. at the 7th Annual meeting of the Environmental Mutogen Society, Atlanta, GA.

Fineterwalder, C. E. (1974), Collaborative Study of the Determination of Polychlorinated Biphenyls in Paperboard, JAOAC 57:518-521.

Firestone, D., Ress, J., Brown, N. L., Barron, R. P., and Damico, J. N. (1972), Determination of Polychlorodibenzo-p-dioxins and Related Compounds in Commercial Chlorophenols, JAOAC 55:85-92.

Fishbein, L. (1974), Toxicity of Chlorinated Biphenyls, Ann Rev of Pharm 14:139-156.

Fishbein, L. (1972), Chromatographic and Biological Aspects of Polychlorinated Biphenyls, J Chromatogr 68, 345-426.

Flick, D. F., O'Dell, R. S., and Childs, V. A. (1975), Studies of the Chick Edema Disease. Similarity of Symptoms Produced by Feeding Chlorinated Biphenyl, Poultry Sci 44, 1460-1465.

Food and Drug Administration Pesticide Analytical Manual, Vol. I Ed. 1968; rev.: July 1969, July 1970, April 1971, Jan 1972, Sept 1972, June 1973, March 1975, July 1975.

Food and Drug Administration, Private Communication (June 8, 1971), Report of Sample No. 4 1970 Interlaboratory Quality Assurance Program (Pesticides in Fish).

Food and Drug Administration, Private Communication (Jan 12, 1973), Sample No. 1 FY/73 Interlaboratory Performance Assurance Program (PCB and Dieldrin in Baby Food).

Friend, M. and Trainer, D. O. (1970), Polychlorinated Biphenyl: Interaction with Duck Hepatitis Virus, Science 170:1314-1316.

Fries, G. F., Marrow, G. S., and Gordon, C. H. (1973a), Long-term Studies of Residue Retention and Excretion by Cows Fed a Polychlorinated Biphenyl (Aroclor 1254), J. Ag Fd Chem 21:117-121.

Fries, G. F., Lillie, R. J., Cecil, H. C., and Bitman, J. (1973b), Retention of Excretion of Polychlorinated Biphenyl Residues by Laying Hens, 165th Meeting of American Chemical Society (Abstract).

Fries, G. E., Marrow, G. S. (1975), Excretion of Polybrominated Biphenyls into the Milk of Cows, Journal of Dairy Science 58:947-951.

Fries, G. S., Cecil, H. C., Bitman, J., and Lillie, R. J. (1976), Retention and Excretion of Polybrominated Biphenyls by Hens, Bull Environ Contam Toxicol, in press.

Fries, G. F., Marrow, G. S., Detering, C. N., Frewitt, L. R., and Cook, R. M. (1975), Distribution of Polybrominated Biphenyl Residues in Tissues of Dairy Cattle, J Diary Sci 58:764 (Abstract).

Fumiko, K. (1972), Estradiol-potentiating Action of PCB (Polychloro-Biphenyl) of PCB (Polychlorobiphenyl), Fukucku-Igaku-Zasshi 63:374-377.

Gardner, A. M., Chen, J. T., Rosch, A. G., and Ragelis, E. P. (1973), Polychlorinated Biphenyls: Hydroxylated Urinary Metabolites of 2,5,2',5'-Tetrachlorobiphenyl Identified in Rabbits, Biochem Biophys Res Comm 55:1377-1384.

Garthoff, L. H., Friedman, L., Hurley, N., Farber, T. M., Peters, E. L., Locke, K. K., Green, S., Sobotka, T. J., Moreland, F., Keys, J., Scalera, J., Rothlein, J., Taylor, M. J., Story, G., Graham, C. H., Marks, E., Cerra, F., and Sporn, E. M. (1975), Biochemical and Cytogenetic Effects Caused by Ingestion of Aroclor 1254 or Firemaster BP-6. Testimony of Dr. A. C. Kolbye at Polybrominated Biphenyl Conference, Michigan Dept. Agric., Lansing, Michigan, May 29.

Giam, C. S. and Wong, M. K. (1972), Problems of Background Contamination in the Analysis of Open Ocean Biota for Chlorinated Hydrocarbons, J. Chromatogr 72:283-292.

Goldstein, J. A., Hickman, P., and Jue, D. L. (1974), Experimental Hepatic Porphyria Induced by Polychlorinated Biphenyls, Toxicol Appl Pharmacol 27:437-448.

Goldstein, J. A., Hickman, P., and Jue, D. L. (1975), Experimentel Hepatic Porphyria Induced by Polychlorinated Biphenyls, Toxicol Appl Pharmacol 27:437-448.

Goldstein, J. A., Hickman, P., Burse, V. W., Bergman, J. (1975), Comparative Study of Two Polychlorinated Biphenyl Mixtures (Aroclor 1242 and 1016) Containing 42% Chlorine on Induction of Hepatic Porphyria and Drug Metabolizing Enzymes, Toxicol Appl Pharmacol 32:461-473.

Goldstein, J. A., McKinney, J. D., Lucier, G. W., Hickman, P., Bergman, H., and Moore, J. A., Toxicological Assessment of Hexachlorobiphenyl Isomars and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. II. Effects on Drug Metabolism and Porphyrin Accumulation, Toxicol Appl Pharmacol, in prees.

Good, C. M. and Pensky, N. (1943), Halowax Acne ("Cable Rash"), Arch Dermatol Syphilol 48(3):251-257.

Goto, M., Sakaguchi, K. and Ogawa K. (1969), Hydropericardium Assay of Rice Oil which Caused Yusho and of Kenechlor 400 in Chickens, Fukuoka Acta Medica 60:131-136.

Grant, D. L., Phillips, W. E. J., and Villeneuve, D. C. (1971a), Metabolism of a Polychlorinated Biphenyl (Aroclor 1254) Mixture in the Rat, Bull Environ Contam Toxicol 6:102-112.

Grant, D. L., Villeneuve, D. C., McCully, K. A., and Phillips, W. E. J. (1971b), Placental Transfer of Polychlorinated Biphenyls in the Rabbit, Environ Physiol 1:61-66.

Grant, D. L., and Phillips, W. E. J. (1974), The Effect of Age and Sex on the Toxicity of Aroclor 1254, Bull Environ Contam Toxicol 12:145-152.

Grant, D. L., Mes, J., and Frank, R. (1975), PCB Residues in Human Adipose Tissue and Milk, Nat Conference on Polychlorinated Biphenyls, Chicago.

Green, S., Sauro, F. M., and Friedman, L. (1975), Lack of Dominant Lethality in Rats Treated with Polychlorinated Biphenyls, Fd Cosmet Toxicol 13:507-510.

Green, S., Carr, J. V., Pahner, K. A., and Oswald, E. J. (1975), Lack of Cytogenetic Effects in Bone Marrow Spermatogonial Cells in Rats Treated with Polychlorinated Biphenyls, Bull Environ Contam Toxicol 13:14-22.

Greenberg, L., Mayers, M. R., and Smith, A. R. (1939), The Systemic Effects Resulting from Exposure to Certain Chlorinated Hydrocarbons, J Ind Hyg Toxicol 21(2):29-38.

Grote, W., Schmoldt, A., Benthe, H. F. (1975), Hepatic Porphyrin Synthesis in Rats after Pretreatment with Polychlorinated Biphenyls, Acta Pharmacol Toxicol 36, 215-224.

Gregory, N. L. (1968), J Cham Soc 13:295.

Hannan, E., Bills, D., and Herring, J. (1973), Analysis of Polychlorinated Biphenyls by Gas Chromatography and Ultraviolet Irradiation, J Agric Food Cham 21:87-90.

Hansell, M. M. and Ecobichon, D. J. (1974), Effects of Chemically Pure Chlorobiphenyls on the Morphology of Rat Liver, Tox and Appl Pharm 28:418-427.

Hansen, L. G., Byerly, C. S., Metcalf, R. L., Bevill, R. F. (1975), Effect of a Polychlorinated Biphenyl Mixture on Swine Reproduction and Tissue Residues, Am J Vet Res 36(1):23-26.

Hara, I. (1969), Health Supervision of Workers Exposed to Chlorobiphenyl in an Electric Condenser Factory, Proc Osaka Prefect Inst Pub Health Ed., Industry Health 7:26-31. In Japanese.

Hara, I., Harada, A., Kumura, S., Endo, T. and Kawano, K. (1974), Follow-up Health Examination in an Electric Condenser Factory after Cessation of PCBs Usage (1st Report), Japan J Ind Health 16:365-366. In Japanese.

Hasegawa, J., Sato, M., and Tsuruta, H. (1973), Report on Survey of Work Area Environmental where PCB is Handled and of the Health Workers Handling PCB. Ministry of Labor, Bureau of Labor Standards and Institute of Industrial Health, Republic of Japan. In Japanese.

Hasegawa, H., Sato, M., and Tsuruta, H. (1973), An Investigation on the Toxicity of Some New Substances Used as PCB Replacement, Concentrations in Air of SAS, KMC-oil and PCBs in Carbonless Paper Producing Plants and Health Examination of Workers, Special Research Report 141-211, Research Coordination Bureau, Science and Technology Agency, Republic of Japan. In Japanese.

Hashimoto, I. (1974), Liver Function Test Results of PCB Influence Checkup, Jap J Ind Health 47:264-269. In Japanese.

Hass, J. R., Chae, K., and McKinney, J. D., unpublished observations.

Hearing Clerk, Dept. HEW (Feb. 1973), Analytical Methodology for Polychlorinated Biphenyls.

Hesse, J. L. (1975), Polychlorinated Biphenyl Usage and Sources of Loss to the Environment in Michigan, Nat Conference on Polychlorinated Biphenyls, Chicago.

Hill, E. F., Heath, R. G., Spann, J. W., and Williams, J. D. (1974), Polychlorinated Biphanyl Toxicity to Japanese Quail as Related to Degree of Chlorination, Poultry Science 53(2):597-604.

Hirayama, C., Okumura, M., Nagi, J., Masuda, Y. (1974), Hypobilirubinaemia in Patients with Polychlorinated Biphenyls Poisoning. Clin Chem Actn 55:97-100.

Hodge, H. C., and Sterner, J. H. (1949), Tabulation, Toxicity Classes, Am Indust Hygiene Assoc Quarterly 10:93.

Holden, A. V. and Meraden, K. (1969), Single Stage Cleanup of Animal Tissue Extracts for Organochlorine Residue Analysis, J. Chromatogr 44:481-492.

Holmes, D. C. and Walden, M. (1972), A Simple Differentiation of Polychlorobiphenyls from Chlorinated Naphthalenes, J Chromatogr 71, 562-563.

Horwitz, W., Ed, (1975), Official Methods of Analysis of the Association of Official Analytical Chemists, 12th Ed, Section 29.001-29.018.

Hubbard, H. L. (1964), Chlorinated Biphenyl and Related Compounds, in "Encyclopedia of Chemical Technology," 2nd Ed, 5:289-298.

Humphrey, H. E. B., and Hayner, N. S. (1975), Polybrominated Biphenyls. An Agricultural Incidence and Its Consequences. An Epidemiological Investigation of Human Exposure, Trace Metals Conference, University of Missouri, Summer.

Humphrey, H. E. B., Price, H. A., and Budd, M. L. (1976), Evaluation of Changes of the Level of Polychlorinated Biphenyls (PCBs) in Human Tissue, Final Report of FDA Contract No. 223-73-2209.

Hurst, J. G., Newcomer, W. S., and Morrison, J. A. (1974), Effects of DDT, Toxaphene, and Polychlorinated Biphenyl on Thyroid Function in Bobwhite Quail, Poult Sci 53:125-133.

Hustert, K. and Korte, F (1972), Ecological Chemistry. XXXVIII. Synthesis of Polychlorinated Biphenyls and Their Reactions under UV Irradiation, Chemosphere 1:7-10.

Hutzinger, O., Nash, D. M., Safe, S., De Freitas, R. S. W., Norstrom, R. J., Wildish, D. J., and Zitko, V. (1972a), Polychlorinated Biphenyls: Metabolic Behaviour of Pure Isomers in Pigeons, Rats and Brook Trout, Science 178:312-313.

Hutzinger, O., Safe, S., and Zitko, V. (1972), Photochemical Degradation of Chlorobiphenyls (PCBs) Environ Health Perspect 1:15-20.

Hutzinger, O., Safe, S., Wentzell, B. R., and Zitko, V. (1973), Photochemical Degradation of di- and octachlorodibenzofuran, Environ Health Perspectives (exptl issue No. 5), 267-271.

Hutzinger, O., Safe, S., and Zitko, V. (1974a) The Chemistry of PCBs, CRC Press, Cleveland, Ohio 44128.

Hutzinger, O., Jamieson, W. D., Safe, S., Paulmann, L., and Ammon, R. (1974), Identification of Metabolic Dechlorination of Highly Chlorinated Biphenyl in Rabbit Nature 252:698-699.

Ikeda, Y., Horiuchi, S., Yoshimoto, H., Falaya, T., Kawamata, K., Kaneko, T. (1970), Studies on the Toxicity of Hazardous Substances, Special Research Report on Prevention of Outbreak of Yusho and its Diagnosis and Treatment. 74-12T.

INTERDEPARTMENT TASK FORCE ON PCBs. (1972), Washington, DC. Polychlorinated Biphenyls and the Environment.

International Council for the Exploration of the Sea. (1974), Report of Working Group for the International Study of the Pollution of the North Sea and its Effects on Living Resources and their Exploration., Charlottenlund Slot, DK-2920, Denmark, Cooperative Research Report No. 30.

Ito, N., Nagasaki, H., Arai, M., et al. (1973), Histopathologic Studies on Liver Tumorigenesis Induced in Mice by Technical Polychlorinated Biphenyls and its Promoting Effect on Liver Tumors Induced by Benzene Hexachloride, J Natl Cancer Inst 51:1637-1646.

Ito, N., Nagasaki, H., Arai, M., Makiura, S., Sugihara, S., and Hirao, K. (1973), Histopathologic Studies on Liver Tumorigenesis Induced in Mice by Technical Polychlorinated Biphenyls and its Promoting Effect on Liver Tumors Induced by Benzene Hexachloride, Journal of the Natl Cancer Institute 51:1637-1642.

Iverson, F., Villeneuve, D. C., Grant, D. L., and Hatina, G. V. (1975), Effect of Aroclor 1016 and 1242 on Selected Enzyme Systems in the Rat, Bull Environ Contam Toxicol 13:456-463.

Jackson, T. F., Halbert, M. S. (1974), A Toxic Syndrome Associated with the Feeding of Polybrominated Biphenyl Contaminated Protein Concentrate to Dairy Cattle, JAVMA 65(5):437-439.

Jansson, B., Jensen, S., Olsson, M., Renberg, L., Sundstrom, G., and Vaz, R. (1975), Identification by GC-MS of Phenolic Metabolites of PCB and p,p'-DDE Isolated From Baltic Guillemot and Seal Ambio 4(2):93-97.

Jefferies, D. J., and Parslow, J. L. F. (1972), Effect of One Polychlorinated Biphenyl on Size and Activity of the Gull Thyroid, Bull Environ Contam Toxicol 8:306-310.

Jelinek, C. F. and Corneluissen, P. E. (1975), Levels of PCBs in the U.S. Food Supply. Nat Conference on Polychlorinated Biphenyls, Chicago.

Jensen, S., Renberg, L., and Olsson, M. (1972), PCB Contamination from Boat Bottom Paint and Levels of PCB in Plankton Outside a Polluted Area, Nature 240:358-360.

Jensen, S. and Sundstrom, G. (1974), Structures and Levels of Most Chlorobiphenyls in Two Technical PCB Products and in Human Adipose Tissue, Ambio 3:70-76.

Jensen, S. and Sundstrom, G. (1974), Metabolic Hydroxylation of a Chlorobiphenyl Containing only Isolated Unsubstituted Positions-2,2',4,4',5,5'-hexachlorobiphenyl, Nature 251:219-220.

Jerina, D. M. and Daly, J. W. (1974), Arene Oxides: A New Aspect of Drug Metabolism, Science 185:573-582.

Johnstone, G. J., Ecobichon, D. J., and Hutzinger, O. (1974), Effect of Pure Chlorinated Biphenyl Compounds on Hepatic Function in the Rat, Toxicol Appl Pharmacol 28:66-81.

Johnstone, G. J., Ecobichon. J., and Hutzinger, O. (1974), The Influence of Pure Polychlorinated Biphenyl Compounds on Hepatic Function in the Rat, Tox and Appl Pharm 28:66-81.

Jondorf, W. R., Parke, D. V., and Williams, R. T. (1955), Studies in Detoxification. The Metabolism of Halogenobenzenes:1:2:3-, 1:2:4- and 1:3:5-trichlorobenzenes, Biochem J 61:512-521.

Jondorf, W. R., Parke, D. V., and Williams, R. T. (1958), Studies in Detoxification. The Metabolism of Halogenobenzenes:1:2:3:4-, 1:2:3:5- and 1:2:4:5-tetrachlorobenzenes, Biochem J 69:181-189.

Kaiser, K., "On the Optical Activity of PCBs." (1974), Environ Pollut 7:93-101.

Karppanen, E. and Kolho. (1974), The Concentration of PCB in Human Blood and Adipose Tissue in Three Different Research Groups. National Swedish Environmental Protection Board Publications 1974:4E: PCB Conference II. Vallingby, Sweden.

Kasza, L., Haberman, B., Brown, C., Capen, C., Trump, B., Hayes, E., Gilmore, C., and Weinberger, M. (1975), FDA Study P-84, PCB and PBB in Rats, (pathology report), October 1.

Keplinger, M. L., et al. (1971), Toxicological Studies with the Chlorinated Biphenyls, Proceedings of the NIEHS Polychlorinated Biphenyl Meeting, Dec. 20-21.

Keplinger, M. L., Francher, O. E., and Calandra, J. C. (1972), Toxicologic Studies with Polychlorinated Biphenyls, Toxicol Appl Pharmacol 19:402-403.

Karst, A. (Sept 23, 1974), A Report to the Michigan Environmental Review Board, Michigan Chemical Corp.

Kihlstrom, J. E., Orberg, J., Lundberg, C., Danielsson, P. O., and Sydhoff, J. (1973), Effects of PCB on Mammalian Reproduction. PCB Conference II, National Swedish Environmental Protection Board Publications 4E:109-112.

Kihlstrom, J. E., Lundberg, C., Orberg, J., Danielsson, P. O., and Sydhoff, J. (1975), Sexual Functions of Mice Neonatally Exposed to DDT or PCB, Env Physiol Biochem 5:54-57.

Kimbrough, R. D. (personal communication, CDC, HEW, Atlanta, GA 30333).

Kimbrough, R. D., Linder, R. E., and Gaines, T. B. (1972), Morphological Changes in Livers of Rats Fed Polychlorinated Biphenyls, Arch Env Hlth 25:354-364.

Kinter, W. B., Markens, L. S., Janicki, R. H., and Guarino, A. M. (1972), Studies on the Mechanism of Toxicity of DDT and Polychlorinated biphenyl (PCBs) Disruption of Osmoregulation in Marine Fish, Environ Health Perspect 1:169-173.

Kimbrough, R. D., Linder, R. E., Burse, V. W., and Jennings, R. W. (1973), Adenofibrosis in the Rat Liver, Arch Environ Health 27:390-395.

Kimbrough, R. D. (1974), The Toxicity of Polychlorinated Polycyclic Compounds and Related Chemicals, Critical Reviews Toxicol 2(4):445-498.

Kimbrough, R. D. and Linder, R. E. (1974), Induction of Adenofibrosis and Hepatomas of the Liver in balb/cJ mice by Polychlorinated Biphenyls (Aroclor 1254), J Natl Cancer Institute 53:547-552.

Kimbrough, R. D., Squire, R. A., Linder, R. E., Strandberg, J. D., Montali, R. J., and Burse, V. W. (1975), Induction of Liver Tumors in Sherman Strain Female Rats by Polychlorinated Biphenyl Aroclor 1260, J. Natl Cancer Inst 55:1453-1459.

Kimbrough, R. D. (1974), The Toxicity of Polychlorinated, Polycyclic Compounds and Related Chemicals, Critical Reviews in Toxicology 2:445-498.

Kimbrough, R. (1975), Pathological Findings Associated with Chronic Experimental Exposure to PCBs, Nat Conf on Polychlorinated Biphenyls, Chicago, Illinois.

Kimura, N. T. and Baba, T. (1974), Neoplastic Changes in the Rat Liver Induced by Polychlorinated Biphenyl, Genn 64:105-108.

Kitamura, M. (1972), Studies on the Chronic Toxicity. Special Reeearch Report on Prevention of Environmental Pollution by PCB-like Substances. Environ Health Criteria for Polychlorineted bi and terphenyls. Japanese Public Health
Kohanawa, M., Shoya, S., Ogure, Y., Moriwaki, M., and Kawasaki, M. (1969a), Poisoning Due to an Oily By-product of Rice-bran Similer to Chick Edema Diseese. I. Occurrence and Toxicity Test, Nat Inst Anim Health Quart 9:213-219.

Kitamura, M., Tsukamoto, T., Sumino, K. Hayakawa, K., Shibata, T., and Hirano, I. (1973), The PCB Levels in the Blood of Workers Employed in a Condenser Factory, Jap J Ind Health 47:354-355. In Japanese.

Kleinert, S. J. (1975), Sources of Polychlorinated Biphenyls in Wisconsin. In press. Presented at the National Conference on Polychlorinated Biphenyls, Chicago.

Koch, R. B., Desaiah, D., Yap, H. H., and Cutkomp, L. K. (1972), Polychlorinated Biphenyls. Effect of Long-term Exposure on ATPase Activity in Fish, Pimephales Promelas, Bull Environ Contam Toxicol 7:87-92.

Kohanawa, M., Shoya, S., Yonemura, T., Nishimura, K., and Tsushio, Y. (1969b), Poisoning Due to an Oily By-product of Rice-bran Similar to Chick Edema Diseaee II. Tetrachlorodiphenyl as Toxic Substance, Nat Inst Anim Health Quart 9:220-228.

Koller, L. D. and Thigpen, J. E. (1973), Reduction of Antibody to Virus in Polychlorinated Biphenyl-Expoeed Rabbits, Am J Vet Res 34:1605-1606.

Koller, L. D. and Zinkl, J. G. (1973), Pathology of Polychlorinated Biphenyls in Rabbits, Am J Pathol 70:363-373.

Kuratsune, M., Yoshimura, T., Matsuzaka, J., and Yamaguchi, A. (1971), Yuso, a Poisoning Caused by Rice Oil Contaminated with Polychlorinated Biphenyls, HSMHA Health Reports 86:1083-1091.

Kuratsune, M., Yoshimura, T., Matsuzaka, J., and Yamaguchi, A. (1972), Epidemiologic Study on Yusho, a Poisoning Caused by Ingestion of Rice Oil Contaminated with a Commercial Bi and Polychlorinated Biphenyls, Environmental Health Perspectives, (Exptl. Issue, No. 1), 119-128.

Kuratsune, M. (1972), An Abstract of Results of Laboratory Examinations of Patients with Yusho and of Animal Experiments, Environmental Health Perspectives, (Exptl. Issue No. 1), 129-136.

Kuratsune, M., Masuda, Y., and Nagayama, J. (1975), Some of the Recent Findings Concerning Yusho, Nat Conference on Polychlorinated Biphenyls, Chicago.

Kuratsune, M. (1976), Personal Communication.

Kutz, F. W. and Strassman, S. C. (1975), Residues of Polychlorinated Biphenyls in the General Population of the United States, Nat Conference on Polychlorinated Biphenyls, Chicago.

Kutz, F. W. and Yang, H. S. C. (1975), A Note on Polychlorinated Biphenyls in Air, Nat Conference on Polychlorinated Biphenyls, Chicago.

La Rocca, P. T. and Carlson, G. P. (1975), The Inhibitory Activity of Polychlorinated Biphenyls on ATPase Activity, Fed Proc 34:246.

Linear, J. I. and Peakall, D. B. (1973), PCB (Polychlorinated Biphenyls), Pharmacodynamics in the Ring Dove and Early Gas Chromatographic Peak Diminution, Environ Pollut 4:59-68.

Linder, R. E., Gaines, T. B., and Kimbrough, R. D. (1974), The Effect of PCB on Rat Reproduction, Food Cosmet Toxicol 12:63-77.

Litterst, C. L. and Van Loon, E. J. (1972a), Enzyme Induction by Polychlorinated Biphenyls Relative to Known Inducing Agents, Proc Soc Exp Biol Med 141:765-768.

Litterst, C. L., Farber, T. M., Baker, A. M., and Van Loon, E. J. (1972b), Effect of Polychlorinated Biphenyls on Hepatic Microsomal Enzymes in the Rat, Toxicol Appl Pharmacol 23:112-122.

Litterst, C. L., and Van Loon, E. J. (1974), Time-course of Induction of Microsomal Enzymes Following Treatment with Polychlorinated Biphenyl, Bull Environ Contam Toxicol 11:206-212.

Makiura, S., Ace, H., Sugihara, S., eta al. (1974), Inhibitory Effect of Polychlorinated Biphenyls on Liver Tumorigenesis in Rats Treated with 3'-methyl -4 dimethyl aminoazobenzene, N-2-fluorenyl-acetamide and diethyl nitrosamine, J Natl Cancer Inst 53:1253-1257.

Masumoto, H. T. (1972), Study of the Silicic Acid Procedure of Armour and Burke for the Separation of Polychlorinated Biphenyls from DDT and Analogs, JAOAC 55:1092-1100.

Matthews, H. B. and Anderson, M. W. (1975), The Distribution and Excretion of 2,4,5,2',5'-pentachlorobiphenyl in the Rat, Drug Metab Disp 3:211-219.

Matthews, H. B. and Anderson, M. W. (1975), Effect of Chlorination on the Distribution and Excretion of Polychlorinated Biphenyls, Drug Metab Disp 3:371-380.

Matthew, H. B. (1975), PCB Chlorination vs. PCB Distribution and Excretion, Nat Conferance on Polychlorinated Biphenyls, Chicago.

Matthews, H. B. and Tuey, D. B. (1976), The distribution and Excretion of 3,5,3',5'-tetrachlorobiphenyl in the Male Rat. Paper No. 133, Fifteenth Annual Meeting of the Society of Toxicology Atlanta, GA.

McKinnie, J. D., Hass, J. R., and Chae, K. (1975), Metabolism of Pure Hexachlorobiphenyl Isomers in Chicks, Personal Communication.

McKinney, J. D., Chae, K., Gupta, B. N., Moore, J. A., and Goldstein, J. A. (1975), Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. I. Relationship of Chemical Parameters, Toxicol Appl Pharmacol in press.

McKinney, J. D. (1975), Toxicology of Selected Symmetrical Hexachlorobiphenyl Isomers: Correlating Biological Effects with Chemical Structure, Nat Conference on Polychlorinated Biphenyls, Chicago.

McNulty, W. P. (1975), Primate Study, Nat Conference on Polychlorinated Biphenyls, Chicago.

Meigs, J. W., Albom, J. J., and Kartin, B. L. (1954), Chloracne from an Unusual Exposure to Arochlor, JAMA 154:1417-1418.

Melvea, B. and Brandt, I. (1973), The Distribution and Metabolism of Labelled Polychlorinated Biphenyls in Mice and Quails, PCB Conference II. National Swedish Environment Board, Publications 4E:87-90.

MICHIGAN CHEMICAL COMPANY. (1974), Review of Polybrominated Biphenyls, Presented to the Michigan Environmental Review Board, September.

Mieure, J. P., Hicks, O., Kaley, R. G. and Saeger, V. W. (1976), Characterization of Polychlorinated Biphenyls, Nat Conference on Polychlorinated Biphenyls, Chicago.

Moore, J. A., Gupta, B. N., and Vos, J. G. (1976), Toxicity of 2,3,7,8-tetrachlorodibenzofuran, Nat Conference on Polychlorinated Biphenyls, Chicago.

Moron, M., Sundstrom, G. and Wachtmeister, C. A. (1973), Polychlorinated Biphenyls VI. 2,3,7,8-Tetrachlorodibenzofuran, a Critical Byproduct in the Synthesis of 2,2',4,4',5,5'-Hexachlorobiphenyl by the Ullmann Reaction, Acta Chem Scand 27:3121-3122.

Motovchi, M. (1956), Toxicity of Chlorinated Diphenyl, J Sci Labor 32:320. In Japanese.

Mulhern, B., Cromare, E., Reichel, W. L., (1971), Semiquantitative Determination of Polychlorinated Biphenyls in Tissue Samples by Thin Layer Chromatography JAOAC 54:548-550.

Nagayama, J., Masuda, Y., and Kuratsune, M (1975a), Dibenzofurans in Kanechlors, Jap J of Hygiene 30:126-129.

Nagayama, J., Masuda, Y., and Kuratsune, M. (1975b), Chlorinated Dibenzofurans in Kanechlors and Rice Oils Used by Patients with Yusho, Fukuoka, Acta Medica 66:593-599.

Nalson, J. A. (1974), Effects of dichlorodiphenyltrichloroethane (DDT) Analogs and Polychlorinated Biphenyls (PCB) Mixtures on 17 Beta-estradiol-3H to Rat Uterine Receptor, Biochem Pharmacol 23, 447-451.

NIEHS Conference on the Toxicity of the Chlorinated Dibenzo-p-dioxins and Dibenzofurans (1973) Research Triange Park, N.C., April 2-3; Environmental Health Perspective 5.

Nishiyama, K., Yano, H., and Kamano, M. (1973), Determination Method for Polychlorobiphenyl in Air and Its Vaporization from Non-carbon Copy Paper, Shikoku Acta Med 29(4):305-310. In Japanese.

Niehizumi, M. (1970), Light and Electron Microscope Study of Chlorobiphenyl Poisoning, Arch Environ Health 21:620-632.

Norris, J. M., Ehrmantraut, J. W., Gibbons, C. L., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., Jewett, G. L., Crummett, W. B., Gehring, P. J., Tirsell, J. B., and Brosier, J. S. (1973), Toxicological Environmental Factors Involved in the Selection of Decabromodiphenyl Oxide as a Fire Retardant Chemical, Applied Polymer Symposium No. 22:195-219.

Norris, J. M., Ehrmanteant, J. W., Gibbons, G. L., Kociba, R. J., Schwertz, B. B., Rose, J. Q., Humiston, G. G., Jewett, G. L., Crummette, W. B., Gehring, P. J., Tireell, J. R., Brosier, J. S., (1974), Toxicoloigical and Environmental Factors Involved in the Selection of Decabromodiphenyl Oxide as a Fire Retardant Chemical, J Fire Flammability/Combustion Toxicology 1:42.

Nowicki, H. G. and Norman, A. W. (1972), Enhanced Hepatic Metabolism of Testosterone, 4-Androstene-3, 17 dione, and 17 Beta-Estradiol in Chickens Pretreated with DDT or Polychlorinated Biphanyle, Steroids 19:85-99.

Oeme, T. (1975), Foreward. The Fifth Reports of the Study on "Yusho" and PCB (Polychlorinated Biphenyls), Fukuoka Acta Medica 55:547.

Orberg, J., Johansson, N., Kilstrom, J. E., and Lundberg, C. (1972), Administration of DDT and PCB Prolongs Estrous Cycle in Mice, AMBIO 1:148-149.

Orberg, J. and Kihlstrom, J. E. (1973), Effects of Long-term Feeding of Polychlorinated Biphenyls (PCB, Clophen A60) on the Length of the Oestrous Cycle and on the Frequency of Implanted Ova in the Mouse, Env Res 6:176-179.

Orberg, J. and Lundberg, D. H. (1974), Some Effects of DDT and PCB on the Hormonal System in the Male Mouse, Environ Physiol Biochem 4:116-120.

Oregon State University (1975), Summary of Research on PCBs, Experimental Health Sciances Center, Oregon State University (NIEHS Grant ES 00210, November, Page 10.)

Ouw, K. H., Simpson, G. R., and Siyali, D. S. (1974), The Use and Health Effects of Aroclor 1242, a Polychlorinated Biphenyl in an Electrical Industry in N.S.W., Australia. Report. Div. of Occup. Health and Rad. Control Health commission of New South Wales, Australia.

Panel on Hazardous Substances (1972), Polychlorinated Biphenyls, Environmental Impact, Environ Research 5:249-362.

Pardini, R. S. (1971), Polychlorinated Biphenyls (PCB), Effect on Mitochondrial Enzyme Systems, Bull Environ Contam Toxicol 6:539-545.

PCB's. (1976), Polychlorinated Biphenyls (PCBs) in Certain Freshwater Fish, Federal Register 1(39):8409-8410.

Peakall, D. B. and Risebrough, R. W. (1975), PCBs and Their Environmental Effects, CRC Critical Reviews in Environmental Control 5:469-508.

Phillips, W. E. J. (1963), DDT and the Metabolism of Vitamin A and Carotene in the Rat, Can J Biochem Physiol 41:1973-1802.

Phillips, W. E. J. and Hidiroglou, M. J. (1965), Carotenoid and Vitamin A Concentrations in Serum Liver of steer Fed Forages Treated with DDT or MCPA, J Agric Food Chem 13:254-256.

Planonow, N. S., Liptrap, R. M., and Geissinger, H. D. (1972), Distribution and Excretion of Polychlorinated Biphenyls (Aroclor 1254) and their Effect on Urinary Gonadal Steroid Levels in the Boer, Bull Environ Contam Toxicol 7:358-365.

Platonow, N. and Chan, N. Y. (1973), Transplacantal Transfer of Polychlorinated Biphenyls (Aroclor (1254) in a Cow, Vet Record 92:69-70.

Platonow, N. S., and Funnell, H. S. (1971), Anti-androgenic-like Effect of Polychlorinated Biphanyls in Cockerels, Vet Rec 88:109-110.

Platonow, N. S. and Karstad, L. H. (1973), Dietary Effects of Polychlorinated Biphenyls on Mink, Can J Comp Med 37:90-95.

Poland, A. P., Smith, D., Matter, G., and Possick, P. (1971), A Health Survey of Workers in a 2,4-D and 2,3,5-T Plant. Arch Environ Health 22:316-327.

Porter, M. L. and Burke, J. A. (1971), Separation of Three Chlorodibenzo-p-dioxins from Some Polychlorinated Biphenyls by Chromatography on an Aluminum Oxide Column, JAOAC 54:1426-1428.

Reynolds, L. M. (1969), Polychlorobiphenyl (PCB's) and Their Interference with Pesticide Residue Analysis, Bull Environ Contam Toxicol 4:128-143.

Ringer, R. K., Aulerich, R. J., and Zabik, M. (1972), Effects of Diatary Polychlorinated Biphenyls on Growth and Reproduction of Mink, Am Chem Soc Meeting, 12:149.

Risenbrough, R. W., Reiche, P., Herman, S. G., Peakall, D. B., and Kirven, M. N. (1968), Polychlorinated Biphenyls in the Global Ecosystem, Nature (London) 220:1098-1102.

Roach, J. A. G. and Pomerantz, I. H. (1974a), The Findings of Chlorinated Dibenzofurens in a Japanese Polychlorinated Biphenyls Sample, Bull Envir Contam and Tox 12:338-342.

Roach, J. A. G. and Pomerantz, I. H. (1974b), The Finding of Chlorinated Dibenzofurans in Aroclor PCBs of Recent Manufacture, Paper No. 53 presented at 88th Annual Meeting of Assn. of Official Anal. Chemists, Washington, DC, Oct 14-17.

Ruzo, L., Zakik, M., and Schuetz, R. (1974). Photochemistry of Bioactive Compounds: Photoproducts and Kinetics of Polychlorinated Biphenyls, J Agric Food Chem 22:199-202.

Ruzo, L. and Zabik, M. (1975), Polyhalogenated Biphenyls: Photolysis of Hexabromo and Hexachlorobiphenyls in Methanol Solution, Bull Environ Contam Toxicol 13:181-182.

Safe, S., Hutzinger, O., and Jones, D. (1975), The Mechanism of Chlorobiphenyl Metabolism, J Agric Food Chem 23(5):851-853.

Safe, S., Ruzo, L. O., Jones, D., Platonow, N. W., and Hutzinger, O., (1975), The Metabolism of 4-Chlorobiphenyl in the Pig, Can J Physiol Pharmacol 53:392-396.

Safe, S. Platonow, N., and Hutzinger, O. (1975b), The Metabolism of Chlorobiphenyls in the Goat and Cow, J Agr Food Chem 23:259-261.

Sagami, S., Kamata, S., and Yoshida, M. (1973), A Case of Chronic PCB-Intoxication, Nishinihon Hifuka 36(6):694-701. In Japanese.

Sanders, O. T., Zapp, R. L., and Kirkpatrick, R. L. (1974), Effect of PCB (Polychlorinated Biphenyls) Ingestion on Sleeping Times, Organ Weights, Food Consumption, Serum Corticosterone, and Survival of Albino Mice, Bull Environ Contam Toxicol 12:394-399.

Sato, M. and Hasegawa, H. (1974), PCB Concentrations in the Blood of Workers Employed in Carbonless Copying Paper Production, Jap J Ind Health 16:365. In Japanese.

Sawyer, L. D. (1973), Collaborative Study of the Recovery and Gas Chromatographic Quantitation of Polychlorinated Biphenyls in Chicken Fat and Polychlorinated Biphenyl - DDT Combinations in Fish, JAOAC 56:1015-1023.

Schmoldt, A., Benthe, H. F., and Fruehling, R. (1974), Induction of Rat Liver Enzymes by Polychlorinated Biphenyls (PCBs) in Dependence on the Dose and Chlorine Content, Arch Toxicol 32:69-81.

Schulte, E. and Ackar, L. (1974), Identifizierung und Metabolisierbarkeit von Polychlorierten Biphenylen, Natuwissenschaften 61:79-80.

Schwartz, L. (1943), An Outbreak of Halowax Acne ("Cable Rash") Among Electricians, JAMA 122(3):158-161.

Schwartz, L., and Barlow, F. A. (1942), Chloracne from Cutting Oils, Pub Health Rpts 57(47):1747-1750.

Schwartz, L. (1936), Dermatitis from Synthetic Resins and Waxes, Am J Pub Health 26:586-592.

Schwetz, B. A., Norris, J. M., Sparschu, G. L., Rowe, V. K., and Gehring, P. J. (1973), Toxicology of Chlorinated Dibenzo-p-dioxins, Adv Chem Series 120:55-69.

Schwetz, B. a., Keller, P. A., and Gehring, P. J. (1974), The Effect of Purified and Commercial Grade Pentachlorophenol on Rat Embryonal and Fetal Development, Tox and Appl Pharm 28:151-161.

Sharp, C. W., Hunt, D. G., Clements, S. T., and Wilson, W. F. (1974), Influence of Dichlorodiphenyltrichloroethane, Polychlorinated Biphenyls and Anionic Amphiphilic Compounds on Stabiliziation of Sodium and Potassium Activitated Adenosine Triphosphatases by Acidic Phospholipids, Mol Pharmacol 10:119-129.

Simon, N. and Siklosi, C. (1974), Influence of Environmental Factors on Porphyrin Metabolism, Berufs-Dermatosen 22:237-259.

Sinclair, P. R., Granick, S. (1974), Uroporphyrin Formation Induced by Chlorinated Hydrocarbons (Lindane, Polychlorinated Biphenyls, Tetrachlorodibenzo-p-dioxin). Requirements for Endogenous Iron, Protein Synthesis, and Drug Metabolizing Activity, Biochem Biophys Res Commun 61:124-133.

Siasons, D. and Welti, D. (1971), Structural Identification of Polychlorinated Biphenyls in Commercial Mixtures by Gas-Liquid Chromatography, Nuclear Magnetic Resonance and Mass Spectrometry, J. Chromatog. 60:15-32.

Sivalingan, P. M., Yoshida, T., and Inada, Y. (1973), Modes of Inhibitory Effects of Polychlorinated Biphenyls on Oxidative Phosphorylation of Mitochondria, Bull Environ Contam Toxicol 10:242-247.

Smith, F. (1948), U. S. Patant 2,449,088; (1949), Chem Abstr 43: 813.

Smith, L. W., Fries, G. F., and Weinland, B. T. (1973), Utilization of Contaminated Dehydrated Poultry Excreta as Feed by Cows and Polychlorinated Biphenyl Residues in Milk, J Dairy Sci, in press.

Smrek, A., Adams, S., Liddle, J., and Kimbrough, R.: Pharmacodynamics of Mirex in Goats: Effect of Reproduction and Lactation. Submitted to Arch Environ Health.

Societe d'Dlectrochimie, d'Electrometallurgie E+ des Aciaries Electriques d'Ugine (1962) Belgian Patent 613,066; (1962) Chem Abstr 57:16492.

Squire, R. A. and Levitt, H. (1975), Report of Workshop on Classification of Specific Heaptocellular Lesions in Rats, Cancer Research 35:3214-3223.

Stalling, D. L. and Huckens, J. N. (1971), Gas-Liquid Chromatography - Mass Spectrometry Characterization of Polychlorinated Biphenyl (Aroclors) and ^{36}Cl-Labeling of Aroclors 1248 and 1254, JAOAC 54:801-807.

Stalling, D. L. and Mayer, F. L. Jr. (1972), Toxicities of PCBs to Fish and Environmental Residues, Environmental Health Perspectives 1:159-164.

Stalling, D. L., Tindle, R. C., Johnson, J. L. (1972), Cleanup of Pesticide and Polychlorinated Biphenyl Residues in Fish Extracts by Gel Permeation Chromatography, JAOAC 55:32-38.

Street, J. C. and Sharma, R. P. (1975), Alteraction of Induced Cellular and Humoral Immune Responses by Pesticides and Chemicals of Environmental Concern: Quantitative Studies of Immunosuppression by DDT, Aroclor 1254, Carbaryl, Carbofuran, and Methylparathion, Tox Appl Pharm 32:587-602

Street, J. C., Urry, F. M., Wagstaff, D. J., and Blau, A. D. (1969), Comperative Effects of Polychlorinated Biphenyls and Organochlorine Peeticides in Induction of Hepatic Microsomal Enzymes, 158th National Meeting, American Chemical Society, New York, NY.

Sugiura, K., Haltori, M., Baba, M., and Goto, M. (1975), Accumulation and Excretion of PCBs in the Mouse, Chemosphere 4(3):181-188.

Sundstrom, G., Hutzinger, O., and Safe, S. (1976), Identification of 2,2',4,4',5,5'-Hexabromobiphenyl as the Major Component of Flame Retardant FireMaster^{R} BP-6, Chemosphere 5:11-14.

Tas, A. C. and Kleipool, R. J. C. (1972), Characterization of the Components of Technically Polychlorinated Biphenyl Mixtures - II, Bull Envir Contam and Tox 8:32-37.

Trotter, W. (1975), Removing the Interference of BDT and its Analogs in the Analysis for Residues of Polychlorinated Biphenyls, JAOAC 58:461-465.

Trotter, W. J. and Young, S. J. V. (1975), Limitation on the Use of Antimony Pentachloride for Perchlorination of Polychlorinated Biphenyls, JAOAC 58:466-468.

Trotter, W. J. (1976), PCB Analysis of Rice Oil Associated with Yusho, Memo to Frank Cordle, FDA, March 11.

Tsukamoto, H., at al. (1969), The Chemical Studies on Detection of Toxic Compounds in the Rice Bran Oils Used by the Patients of Yusho, Fukuoka, Acta Medica 60:496-512.

Tucker, R. K., and Crabtree, D. G. (1970), Handbook of Toxicity of Pesticides to Wildlife, Bureau fo Sport, Fisheries, and Wildlife, Resources Publ. 84, U.S. Dept. of Interior, Washington D. C.

Turner, J. C., and Green, R. S. (1974), Effect of a Polychlorinated Biphenyl (Aroclor 1254) on Liver MIcrosomal Enzymes in the Male Rat, Bull Environ Contam Toxicol 12:669-671.

Veinio, H. (1974), Enhancement of Microsomal Drug Oxidation and Glucuronidation in Rat Liver by an Environmental Chemical, Polychlorinated Biphenyl, Chem - Biol Interact 9:379-387.

Van Miller, J. P., Hsu, I. C., and Allen, J. R. (1975), Distribution and Metabolism of ^{3}H-2,5,2',5-tetra-chlorobiphenyl in Rats, Proc Soc Exp Biol Med 148:682-687.

Veith, G. D. (1975), Baseline Concentrations of Polychlorinated Biphenyls and DDT in Lake Michigan Fish, 1971, Pest Monitoring Journal 9:21-29.

Villeneuve, D. C., Grant, D. L., Phillips, W. E. J., Clark, M. L., and Clegg, D. J. (1971a), Effects of PCB (Polychlorinated Biphenyl: Aroclors 1221 and 1254), Administration on Microsomal Enzyme Activity in Pregnant Rabbits, Bull Environ Contam Toxicol 6:120-128.

Villeneuve, D. C., Grant, D. L., and Phillips, W. E. J. (1972), Modification of Pentobarbital Sleeping Times in Rats Following Chronic PCB Ingestion, Bull Environ Contam Toxicol 7:264-269.

Villeneuve, E. C., Jennings, R. W., Burse, V. W., and Kimbrough, R. D. (1974), Evidence of Chlorodibenzo-p-dioxin and Chlorodibenzofuran in Hexachlorobenzene, J Agr Food Chem 22:916-917.

Villeneuve, D. C., Grant, D. L., Khare, K., Clegg, D. J., Beer, H., and Phillips, W. E. J. (1971b), The Fetotoxicity of a Polychlorinated Biphenyl Mixture (Aroclor 1254) in the Rabbit and in the Rat, Env Physiol 1:67-71.

Vos, J. G. and Koeman, j. H. (1970), Comparative Toxicologic Study with Polychlorinated Biphenyls in Chicken with Special Reference to Porphyrie, Edema Formation, Liver Necrosis, and Tissue Residues, Tox Appl Pharm 17:656-668.

Vos, J. G., Koeman, J. J., Van der Maas, H. L., Ten Noever de Breuw, M. C., and De Vos, R. H. (1970), Identification and Toxicological Evaluation of Chlorinated Dibenzofuran and Chlorinated Nephthalene in Two Commercial Polychlorinated Biphenyls, Fd Cosmet Toxicol 8:625-633.

Vos, J. G. and Beems, R. B. (1971), Dermal Toxicity Studies of Technical Polychlorinated Biphenyls and Fractions Thereof in Rabbits, Tox Appl Pharm 19:617-633.

Vos, J. G. (1971), Polychlorinated Biphenyls as Inducers of Hepatic Porphyria in Japanese Quail with Special Reference to -Aminolevulinic Acid Synthessse Activity, Fluorescence, Acid Residues in Liver, Toxicol Appl Pharmacol 20:232-240.

Vos, J. G. and Notenboom-Ram, E. (1972), Comparative Toxicity Study of 2,4,5,2',4',5'-Hexachlorobiphenyl and a Polychlorinated Biphenyl Mixture in Rabbits, Tox and Appl Pharm 23:563-578.

Vos, J. G. (1972), Toxicology of PCB's for Mammals and for Birds, Env Hlth Persp 1:105-117.

Vos, J. G. and VanGenderen, H. (1973), Toxicological Aspects of Immuno-suppression. Aspects of Immunosuppression Published in Pesticides in the Environment, a Continuing Controversy, Ed. by W. B. Deichman, (8th International Conference on Toxicology and Occupational Medicine, Miami), Published by Intercontinental Med. Book Co., New York, NY.

Vos, J. G. and DeRoij, T. L. (1972), Immunosuppressive Activity of a Polychlorinated Biphenyl Preparation on the Humoral Immune Response in Guinea Pigs, Tox Appl Pharm 21:549-555.

Walker, C. R. (1975), Pre-1972 Knowledge of Non Human Effects of Polychlorinated Biphenyls, Nat Conf on Polychlorinated Biphenyls, Chicago, Illinois.

Wasserman, D., Wasserman, M. (1972), Ultrastructure of Adrenal Zona Fasciculata in Polychlorinated Biphenyls Receiving Rats, 17th Intern Congress Occ Health, Buenos Aires.

Wasserman, D., Wasserman, M., Cucos, S., and Djavaherian, M. (1973), Function of Adrenal Glad-zone Fasciculata in Rats Receiving Polychlorinated Biphenyls, Environ Res 6:334-338.

Webb, R. G. and McCall A. C. (1972), Identities of Polychlorinated Biphenyl Isomers in Aroclors, JAOAC 55:746-752.

Webb, R. G. and McCall, A. C. (1973), Quantitative PCB Standards for Electron Capture Gas Chromatography, J Chromatogr Sci 11:366-373.

Weingarten, H. (1961), Steric Effects in the Gomberg Reaction, J Org Chem 26730-733.

Welborn, J. A., Senator and Chairman, A Report from the State Senate Special Investigating Committee, The Contamination Crisis in Michigan, July, 1975.

WHO Task Group (1975), Environmental Health Criter for Polychlorinated Biphenyls and Terphenyls.

Wit, J. G. (1972), Enzyme Induction with Specific Reference to Chemical Porphyria, Third Technical Meeting on Occurrence and Significance of Chemicals in the Environment, Berlin, Germany.

Wong, R. G., Nowicki, H. G., and Norman, A. W. (1974), Effects of Polychlorinated Biphenyls on Calciferol (Vitamin D) Mediated Calcium Metabolism, Pestic Biochem Physiol 4:170-177.

Yamamoto, H., and Yoshimura, H. (1973), Metabolic Studies on PCBs. III. Complete Structure and Acute Toxicity of the Metabolites of 2,4,3'4'-tetrachlorobiphenyl, Chem Pharm Bull 21:2237-2242.

Yamamoto, H. (1974), Statistics of Occupational Disease (Result of Special Medical Examination), New Occupational Health Handbook 1010-1013, The Institute for Science of Labour, In Japanese.

Yap, H. H., Desaiah, D., Cutkomp, L. K., and Koch, R. B. (1971), Sensitivity of Fish atpase to Polychlorinated Biphenyls, Nature (London) 233:61-62.

Yobs, A. R. (1972), Levels of Polychlorinated Biphenyls in Adipose Tissue of the General Population of the Nation, Environ Health Perspectives 1:79-81.

Yushimura, T. (1971), Epidemiological Analysis of "Yusho" Patients with Special Reference to Sex, Age, Clinical Grades and Oil Consumption, Fukuoka, Acta Medica 62:104-108.

Young, S. and Burke, J. (1972), Micro Scale Alkali Treatment for Use in Pesticide Residue Confirmation and Sample Cleanup, Bull Environ Contam Toxicol 7:160-167.

Zepp, R. L. Jr., Sanders, O. T., and Kirkpatrick, R. L. (1974), Reduction of Pentobarbital-Induced Sleeping Times in PCB (Polychlorinated Biphenyl)-Treated Cottontail Rabbits, Bull Environ Contam Toxicol 12:518-521.

Zitko, V., Hutzinger, O., and Choi, P. M. K. (1972), Contamination of the Bay of Fundy - Gulf of Maine Area with PCBs, PCTs, Chlorinated DBF and DBD, Environ Health Perspect 1:47-50.

Zitko, V., Hutzinger, O., and Safe, S. (1971), Retention Times and Electron Capture Detector Responses of Some Individual Chlorobiphenyls, Bull Environ Contam and Toxicol 6:160-163.

Zitko, V., Hutzinger, O., and Safe, S. (1971), Retention Times and Electron-capture Detector Responses of Some Individual Chlorobiphenyls, Bull Environ Contam Toxicol 6:160-163.

Zitko, V., Hutzinger, O., and Choi, P. M. K. (1974), Determination of Pentachlorophenol and Chlorobiphenylols in Biological Samples, Bull Environ Contam Toxicol 12:649-653.

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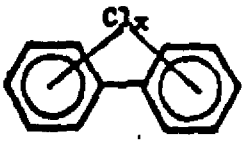
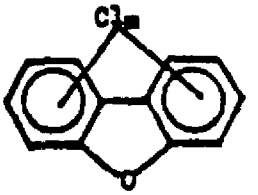
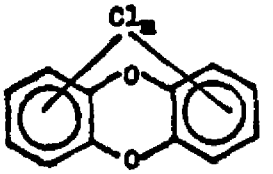
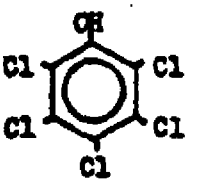
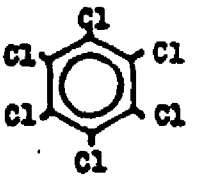
Appendix A
CHLORINATED AROMATIC COMPOUNDS
REFERRED TO IN CHEMISTRY REPORT

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