NS 296

TECHNICAL REVIEW OF THE HEALTH EFFECTS OF PCBs

by

Robert C. James, Ph.D.
Morris F. Cranmer, Ph.D.
Raymond D. Harbison, Ph.D.

August 17, 1981

ecology and environment, inc.
188 SUGG ROAD, P.O. BOX D, BUFFALO, NEW YORK 14225, TEL. 716-632-4491
International Specialists in the Environmental Sciences

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CONTENTS

Executive Summary 1

1. Physical - Chemical Properties 6

2. Production and Uses 7

3. Occurrence 9

3.1. Air. 9

3.2. Water. 9

3.3. Soils. 10

3.4. Marine Organisms. 10

3.5. Fish and Other Aquatic Organisms. 10

3.6. Birds. 10

3.7. Man. 11

3.8. Food. 11

3.9. Summary of Occurrence. 11

4. Animal Studies 12

4.1. Pharmacokinetics and Metabolism. 12

4.2. Acute & Subchronic Toxicity. 16

4.3. PCB Contaminants. 17

5. Mutagenicity 18

6. Reproduction 19

7. Birth Defects 20

8. Carcinogenicity 21

8.1. What Mechanisms Provide for the Expression of Carcinogenicity or Oncogenicity? 22

8.2. Comparison of the Characteristics of Oncogens and Effects of PCBs 28
8.3. Comparison of the Characteristics of Carcinogens and the Effects of PCBs 29
8.4. What Has Been Characterized as Cancer In The Bioassay Literature? 30
8.5. Cholangiofibrosis (Adenofibrosis). 31
8.6. Foci or Areas of Cellular Alteration. 31
8.7. Neoplastic Nodules. 32
8.8. Hepatocellular Carcinoma. 34
8.9. What Level of Confidence Can be Placed on Liver Tumors? 35
8.10. PCB Oncogenicity and Related Studies. 37
8.11. Tumor Initiation Modification. 48
8.12. Immunosuppressive Effects on Tumor Cells. 50
8.13. Are Chlorinated Hydrocarbons Different? 51
8.14. Carcinogenicity Summary. 53
8.15. Conclusion. 53
9. Effects of PCBs in Humans 54
9.1. Summary of Human Health Effects. 59
10. Summary and Conclusion 60
11. List of Tables 61
Table 1 - Isomers of PCBs
Table 2 - Physical Properties of Commercial PCBs
Table 3 - End Uses of Aroclors by Type
Table 4 - PCBs in Food
Table 5 - Toxicity Rating Chart
Table 6 - Actual Toxicity and the Toxicity Rating Chart of Some Selected

Chemicals

Table 7 - General Symptoms of "Yusho" Patients

Table 8 - Tumorigenic Effects of PCBs

12. List of Figures

Figure 1 - Chemical Structure of PCB

References

Glossary

Biographical Information on the Authors

Contents

EXECUTIVE SUMMARY

The PCB animal toxicity literature documents that acute exposures to PCBs produce relatively unremarkable acute toxic responses. Utilization of standardized criteria results in PCBs acute toxicity being classified as only slightly toxic to non-toxic (Table 3). This ranking is based in part on the fact that doses required to produce short term harmful effects in some test species are so high that a dose predicted to produce similar effects in a man would exceed 1 quart. However, as is often the case with many other halogenated organic chemicals, additional concern is justifiable because of ubiquitous distribution (Table 4) and potential to cause long-term effects with fractions of the individual doses necessary to produce acute exposure. The disproportionate concern with long-term low-level exposures is primarily due to biological and ecological persistence and accumulation in the body fat.

The primary human health and environmental concerns relate to long-term, low level exposures. The total spectrum of animal and human toxic responses must be considered and related to anticipated human exposure before the extent of hazards associated with any given exposure to PCBs can be estimated or documented. All available data on the toxicity of PCBs to animals have been reviewed and compared with carefully documented cases of human exposure. A pattern of comparable, comparative toxicity emerges. Mutual expression of most human and animal responses enhances the relevance of comparative toxicological conclusions and generalizations which are necessary before extrapolating from past experience to possible hazards or margins of safety associated with future exposure to

MONS 016037

PCBs. The following paragraphs briefly summarize the data reviewed and those components of the data emphasized in the eventual estimation of the hazard posed by exposure to PCBs.

The effects of PCBs can include acneform lesions, hair loss, a lowered immune response, liver damage and death (Table 7). The severity of the observed responses is not unusual or inconsistent with toxic doses of most chemicals and unremarkable when compared to many useful chemicals or drugs.

PCBs yield negative mutagenic results in the Ames Test (measure of mutagenic potential at the gene level) and the dominant lethal test (test for heritable damage leading to embryotoxicity and birth defects), and are not clastogenic (gross, morphological damage to chromosomes) to blood or sperm cells of rats or cultured human blood cells. PCBs must be assumed to be nonmutagenic.

PCBs have produced embryotoxicity and spontaneous abortions only at doses clearly producing maternal toxicity and are no more toxic to the fetus than most chemicals when given at toxic doses to the maternal animal. PCBs can induce fetal liver microsomal enzymes. PCBs produce minimal changes in newborns and are much less toxic than some common vitamins and hormones.

The carcinogenic potential of PCBs is debatable because one test result is inconsistent and contradictory with all others (Table 8). Results in the female rats in one of the many studies reported was interpreted as associating an increase in liver cancer with PCB treatment. Two other chronic tests in the same species, the rat, as well as tests in the mouse, did not reveal a statistical, significant increase in liver cancer with PCB treatment. The most recent test,

performed by the National Cancer Institute, did not demonstrate that PCBs produce cancer in the rat. The results of all chronic tests are compatible only if the assumption is made that the females of the Sherman rat strain are unusually susceptible to a single PCB (Aroclor 1260). A sex-specific sensitivity is documented, since only the females and not the males of the Sherman rat strain developed liver cancer; therefore, the females of this strain of rat must be more susceptible than the males. In addition, a strain difference exists because, in addition to the male Shermans, both sexes of two other strains of rat did not develop liver cancer as a result of exposure to PCBs. A species difference is documented, since neither sex of several strains of mice produce positive results. A compound-specific sensitivity may also exist in rats, since only the most completely chlorinated PCB (Aroclor 1260) tested produced liver cancer, while the less chlorinated PCB (Aroclor 1254) did not in either sex. It should be noted that the PCBs that were interpreted to induce liver cancer in the Sherman strain rat were likely contaminated with unknown amounts of the highly toxic chlorinated dibenzofurans. The possibility that polychlorinated dibenzofurans influenced the only positive test cannot be ignored. Finally, it must be emphasized that the criteria utilized in diagnosing the cancer in the female Sherman rats is controversial.

Human data reveal that PCBs produce unremarkable toxic effects. A mass poisoning episode in Yusho, Japan produced chloracne, excessive discharge from the eyes, irregular menstruation, and high blood triglyceride levels but no evidence of substantial liver damage (Table 7). The PCB at Yusho contained exceptionally high levels of polychlorinated dibenzofurans. Studies over the last decade of workers occupationally exposed to PCBs provide evidence of minimal toxicity, primarily skin problems, and occasional minimal changes in liver function.

MONS 016039

There are no data substantiating an excess cancer risk after 50 years of PCB use. However, the question of PCBs' carcinogenic potential in humans will remain a theoretical possibility for some time because the latency period at low levels could approach man's normal lifespan. The largest study to date, with 2,500 employees from two capacitor factories, did not demonstrate any causal relationship between exposure to PCBs and any excess cancer.

A thoughtful evaluation of the hazard potential of PCBs must review, analyze, and relate all the relevant evidence available. Almost all residents of industrialized countries contain detectable levels of PCBs in their bodies and several ppm have been consistently measured in the body fat of "normal" people. Measurements of blood PCB levels in "normal" workers in PCB manufacturing plants reveal that blood levels may be hundreds to thousands of times higher than the average population. Thus, it appears that small doses of PCBs obtained from our environment and food supply are generally tolerated. The toxicological data indicate that concern for hazard at high levels of exposures to PCBs should be no greater and often less than for many other common industrial chemicals.

PCBs were once a useful chemical in our society. Uncontrolled waste disposal practices resulted in human exposures and environmental contamination. Since PCBs were widely dispersed and are persistent, they became ubiquitous in our environment. Concern over adverse health effects are largely due to the possibility of long-term exposure to PCBs. Drastic regulatory measures were considered necessary in 1977 due to the then existing enormous scientific uncertainty surrounding health effects. Compounding this uncertainty is the effect that all PCBs do not present the same hazard because they are different compounds (Table 1). The toxicity of PCBs varies greatly with the degree of chlo-

MONS 016040

rination and extent of contamination with polychloro dibenzofurans. Analysis of the toxicological literature leads to conclusions that support the view that PCBs have not been demonstrated to be hazardous under most conditions of exposure to the public and that intermittent, miniscule exposures are not likely to lead to disasterous consequences.

MONS 016041

TECHNICAL REPORT

MONS 016042

1. PHYSICAL - CHEMICAL PROPERTIES

Polychlorinated biphenyls (PCBs) are formed by chlorinating any one of ten available carbon atoms of the biphenyl molecule (see Figure 1). In the commercial synthesis of PCBs, the biphenyl molecule is chlorinated with anyhydrous chlorine using either ferric chloride or iron fillings as a catalyst (Hutzinger et al. 1974). The chlorination of the biphenyl structure cannot be controlled strictly enough to generate only two pure, stereochemically homologous products. Thus, the commercial preparations of PCBs are actually mixtures of chlorinated biphenyls with varying chlorine content and chlorine position. Since there are 10 available carbons to chlorinate on the biphenyl molecule, there are a possible 209 different PCB isomers that can be generated, as seen in Table 1. An estimated 40-70 different chlorinated biphenyl compounds can be present in each of the higher chlorinated commercial mixtures. For example, Aroclor 1254 contains 69 different molecules that differ in the number and position of chlorine atoms. A scheme was developed to identify specific PCB isomers from the many possible choices. The lowest possible numbers are always assigned and the prime numbers are always given to the chlorines on the phenyl ring containing the fewest chlorine atoms (see figure 1). About half of the 209 possible chlorobiphenyls do not occur in any commercial preparations, and the majority of these compounds are those in which only one ring is completely or predominantly chlorinated (Hutzinger et al. 1974, NIOSH 1977). For example, there are no penta-, hexa-, or heptachlorobiphenyl isomers in which one ring is fully chlorinated, nor are there penta or hexachloro derivatives containing four chlorine atoms on one ring.

PCBs have unique physical and chemical properties that have made them useful and

applicable to many commercial needs. These properties include thermal stability; resistance to oxidation by acids, bases, and other chemical agents, excellent electrical insulation; fire resistance; and low volatility A partial list of some pertinent chemical properties has been provided for a few PCB mixtures in Table 2.

2. PRODUCTION AND USES

The synthesis of PCBs has been known for over 100 years. It was first prepared in 1867 by Griefs, who heated biphenylbis (diazonium) platinum chloride with sodium carbonate (IARC 1978). PCBs were first produced in 1929 by the Swann Chemical Company, which was then purchased in 1935 by the Monsanto Company (Monsanto 1979). By 1964, there were at least six U.S. companies with registered trademarks for commercial brands of PCBs (IARC 1978). Commercial PCB products have also been produced by Great Britain, Japan, Germany, France, Italy, Spain, Czechoslovakia, Poland, the USSR, India, Brazil and Argentina (NIOSH 1977, IARC 1978). It has been estimated that over 1 billion pounds of PCBs were sold in North America alone (IARC 1978, Monsanto 1979) and they have been in use in almost every industrialized nation of the world.

The physical properties of PCBs (as mentioned above) were applicable to many industrial situations and prompted their use in many commercial areas (see Table 3). Because of their resistance to fire and breakdown by heat combined with their electrical insulating capabilities, they found numerous uses in the electrical industry primarily in capacitors and transformers. They were also found to be useful as lubricants, heat transfer liquids, and hydraulic fluids. Unfortunately, they were also utilized for many "open" applications where their emis-

sions into the environment could not be controlled. Such uses included combinations with plasticizers, inks, surface coatings for wood and cement surfaces, adhesives, pesticide formulations as an extender, carbonless duplicating paper and immersion oils for microscopes (IARC 1978, Monsanto 1979). Decachlorobiphenyl was also imported from Italy into the United States for use as a filler for investment casting waxes.

Because of concern generated in the late 1960's concerning the environmental persistance of PCBs, production of PCBs began to be phased out in the U S. in the early 1970's. In 1971, the sale of PCBs was voluntarily limited to closed system uses by the sole manufacturer at that time, and production was later completely discontinued in 1977.

It is important to note that, even with the cessation of PCB production per se, other environmental sources of PCB may exist. For example, it has been reported that some PCBs are products of DDT photolysis (Plimmer and Kligebeil 1973) and Oyeta et al. (1976) reported the photoformation of PCBs from the sunlight irradiation of mono-, di-, tri-, tetra-, and hexachlorobenzines. Besides these sources of PCBs, Gaffney (1977) reported the formation of various mono-, di-, and trichloro-biphenyls resulting from the final chlorination of municipal wastes containing biphenyl. Laboratory chlorination of influent and effluent from a municipal waste treatment facility also resulted in the formation of these and other chloroorganic substances such as di- and trichlorobenzenes.

3. OCCURRENCE

PCBs were first recognized to be an environmental contaminant in 1966. In the next few years, PCBs were identified in the environment worldwide and found to have become a trace contaminant even in those people not occupationally exposed to them. Since the environmental occurrence of PCBs has been discussed extensively in several articles (Peakall 1975, NIOSH 1977, IARC 1978, Wasserman et al., 1979, EPA 1980) the following summary paragraphs will only include a few examples that illustrate the ubiquitous nature of the PCB contamination problem. For example, PCBs have been found in:

3.1. Air. A Japanese survey reported the the concentration of PCBs in urban air range from 0.002 - 0.02 ng/m^3. A 1975 report on three American cities (Fort Collins, Colorado; Jackson, Mississippi; and Miami, Florida) revealed that the average concentration of PCBs in air was 0.1 ng/m^3. To put these data into perspective, the current government standard for workroom air is only 0.1 ng/m^3. It was estimated in 1975 that approximately 2 million pounds of PCBs are deposited and redistributed in the U.S. yearly as rain and particulate matter.

3.2. Water. It has been estimated that the waters of Lake Michigan contain 10 ng/l of PCBs, while the average concentration measured in seawater from the Mediterranean was 13 ng/l. PCBs in the Hudson River have been measured as high as 2.8 ng/l in the water and 6700 ng/kg in the sediments. Municipal water supplies have been contaminated with up to 20 ng/l of PCBs from paint contaminated with in excess of 1%.

MONS 016046

3.3. Soils. A survey in 1972 revealed that while only 0.1% of the samples taken from agricultural areas contained detectable amounts of PCBs, 63% of similar samples from metropolitan areas had measurable levels.

3.4. Marine Organisms. PCBs have been identified in almost all plants and animals in the Atlantic Ocean. Residues in plankton have been found as high as 1.5 ppm, in mussels of 1.70 ppm, in shrimp of 7.0 ppm, in gray seals of 14.5 ppm, and in whales and dolphins, levels up to 147.0 ppm have been recorded.

3.5. Fish and Other Aquatic Organisms. Fish can bioaccumulate PCBs in the range of tens of thousands to several hundred thousands of times greater than the concentration of PCBs in water. Thus, it is not surprising to find reports where fish from eastern rivers of the United States have had PCBs as high as 140 ppm in their flesh or fish from the Great Lakes have at one time contained levels ranging from 2.7 - 26.0 ppm. Sardines taken from the Mediterranean have had 0.7 - 4.7 ppm, while sardines from other areas such as the Atlantic coast, Adriatic Sea, France and Tokyo Bay had 0.3 - 0.4, 0.3 - 1.06, 0.7 - 5.1 and 1.0 - 11.0 ppm, respectively.

3.6. Birds. The highest concentrations of PCBs for any species are probably those found in birds. Birds are often at the top of food chains and therefore the most often affected by chemicals that can be bioconcentrated. Residues reported in carnivorous, hunting species are as follows: 16,000 ppm in white tailed eagles, 2,000 ppm in peregrin falcons, and 900 ppm in herons. Liver concentrations in gannets of England ranged from 4,720 - 9,590 ppm. Reduction of avian populations by PCBs has been feared because of the excessively high residues measured and the fact that they may affect reproduction by altering the

MONS 016047

eggs themselves, causing neurological changes reducing fitness and defense, and decreasing the immunologic defense against infectious diseases. PCBs have been found in bird eggs at concentrations as high as several thousand ppm, although they are more often reported in the 10-100 ppm range.

3.7. Man. Studies have shown that man is exposed to PCBs through the environment because they have been found in the fat or blood of persons not occupationally exposed. Jelinek and Corneliussen (1976) calculated the daily intake of PCBs in the diet of a teenage male at 8-15 ug/day for 1971-1973. Fat concentrations of the "normal" population can be several ppm with blood levels in the ppb range. PCB levels are usually higher in the male population, and measurable levels of PCBs have been reported in people from the U.S., England, Norway, Finland, Netherland, France, Germany and Japan. Thus, it can be concluded that human PCB contamination from our environment is a worldwide phenomenon.

3.8. Food. Possibly the major source of human exposure to PCBs has been our food. Food may become contaminated in many ways including residues of pesticide formations containing PCBs, migration from packaging made from recycled paper, rainfall and particulate fallout onto crops, and, of course, from fish and meat grown in contaminated areas. The extent of PCB contamination of food has been monitored by the FDA and USDA since 1969. The results of some of the monitoring is presented in Table 4. Current FDA tolerance levels are reported in Table 5. The presence of PCBs in food declined over the years and this decrease correlates with the decline in its industrial use for this period.

3.9. Summary of Occurrence. There has been a sharp curtailment of PCB production and dispersive use applications from a record high of 70 million lbs in

1969. It is believed that it will take many years for ecosystems such as Lake Michigan to be cleaned of the PCBs even if no new input is made. Due to high adsorption coefficient and resistance to degradation, the PCBs have accumulated in quantity in bottom sediments. The final environmental sink for PCBs is predicted to be degradation in the atmosphere and sequestration by irreversible binding to metabolically stagnant sediments of lakes (Neeley 1977).

4. ANIMAL STUDIES

4.1. Pharmacokinetics and Metabolism. It is generally acknowledged that the toxicological assessment of commercially available PCBs has been complicated by the heterogeneity of the chlorobiphenyl mixtures. Marked differences exist in the physical and chemical properties of each specific chlorobiphenyl that may influence the rates of absorption, distribution, biotransformation and excretion.

According to the scheme of Matthews and Kato (1979), PCBs are a Type III class of halogenated aromatic hydrocarbons. As members of this class, one correctly predicts that PCBs would be quite non-polar, readily absorbed orally, and slowly metabolized. PCBs are almost completely absorbed by the gut, having a 92 - 98.9% efficiency of oral absorption in the rat which is largely independent of the degree of chlorination for the dosage range of 5 - 100 mg/kg (Albro and Fishbein 1972). In another study in rats by Matthews and Anderson (1975), it was found that PCBs are taken up from the blood initially by the liver (high perfusion and affinity) and muscle (large percent of the total body mass). PCBs then redistribute to the skin and adipose tissue (highest affinity, low perfusion) such that the tissue concentrations ultimately equilibrate in the following or-

der: adipose tissue > skin > liver > muscle (Matthews and Anderson 1975, Lutz et al. 1977).

The absorption and the distribution patterns of the various chlorinated biphenyls are similar, but metabolism is not. Studies have shown that the monochloro and dichloro biphenyl compounds are extensively metabolized, but that increasing the chlorination decreases the extent of metabolism (Matthews and Anderson 1975). For example, in the rat the monochlorobiphenyl is metabolized 5 times faster than the dichlorobiphenyl, 25 times faster than pentachlorobiphenyl, and 200 times faster than hexachlorobiphenyl (Lutz et al. 1977). Thus, while the mono and dichlorobiphenyls are extensively metabolized and excreted within days, it was calculated that less than 20% of a single 0.6 mg/kg dose of 2, 2', 4, 4', 5, 5' hexachlorobiphenyl would be eliminated during the lifetime of the animal (Matthews and Anderson 1975). The extent of chlorination and the position of the chlorine atoms on the biphenyl ring both significantly alter the rate of metabolism. Studies in mice and rats indicate that the hydroxylation rate increases with the relative availability of adjacent unsubstituted carbon atoms (Tuhoy and Matthews 1977, Mitsutani et al. 1977). For example, in mice the tetrachlorobiphenyls are metabolized at rates such that the accumulation of PCB is 2, 2', 3, 3'=3, 3', 4, 4'<2, 2', 5, 5'<2, 2', 4, 4'<3, 3', 5, 5'. When metabolism of the mice was increased by induction with phenobarbital, the order for accumulation was essentially the same with 2, 2', 5, 5'<2, 2', 4, 4'<3, 3', 5, 5' leaving the authors to conclude that this reflected the recalcitrancy of the particular chlorinated biphenyl to be metabolized (Mitsutani et al. 1977). Similar evidence has been provided in rat studies where adding chlorines at the 4 and 4' positions, thereby eliminating the unsubstituted vicinal carbons of 2,

2', 5, 5' tetrachlorobiphenyl, dramatically lowered the rate of metabolism (Matthews and Anderson 1975, Tuhey and Matthews 1977).

The ability of PCBs to induce hepatic enzymes and thereby increase metabolism has been well documented (Litterst and Van Loon 1972, Litterst et al. 1972, Testa and Jenner 1976, Ecobichon and Comeau 1975, Johnstone et al. 1974 and Goldstein et al. 1977). PCBs are very effective inducing agents whose potency on a molar basis far exceeds that of phenobarbital or DDT and are capable of causing a 100% increase in cytochrome P-450 at the relatively low dose of 5 mg/kg (Litterst and Van Loon 1972). However, the position and degree of chlorination on the biphenyl nucleus still plays an important role in the effects elicited. Ecobichon and Comeau (1975) found that to enhance oxidative metabolism, chlorination at the 4 or 4' position was required. They and others have also reported that increased chlorination increases the amount of induction seen (Litterst et al. 1972, Johnstone et al. 1974 and Goldstein et al. 1974). Goldstein and coworkers (1974) demonstrated that while PCB mixtures can induce both cytochrome P-450 and cytochrome P-448, cytochrome P-450 is always induced by isomers in which chlorines are present at the ortho and para positions regardless of the extent of chlorination, while symmetrically chlorinated biphenyl isomers with chlorines in the meta and para positions only induce cytochrome P-448.

The metabolism of PCBs is also of interest because of its possible relationship to the compound's toxicity. The formation of an arene oxide during the hydroxylation of PCBs has been suggested from studies demonstrating dihydrodiol metabolites and NIH shifts in deuterium and chlorine atoms placed at the para position (Matthews et al., 1978, Daly et al., 1972). Arene oxides of certain polyaromatic hydrocarbons (PAH) are known to bind to nucleic acids and it has

MONS 016051

been postulated that the interaction of these reactive molecules with nucleophilic sites of DNA and other macromolecules leads to the induction of tumors Thus, it has been hypothesized that arene oxide formation of PCBs may also lead to alkylation of critical cellular sites thereby inducing cancer (IARC 1978, EPA 1980, Allen and Norbeck 1973). However, there are several differences between PCBs and PAHs that should also be considered when suggesting such a mechanism For example, while there is a good correlation between the mutagenicity of PAHs in the Ames assay and their carcinogenicity in rodents, such is not the case for PCBs. Only monochlorobiphenyl is positive in the Ames assay, while the polychlorinated biphenyls were not (Wyndham et al. 1976). Here it appears that the decreased metabolism with increased chlorination would reduce the likelihood of formation of a reactive metabolite necessary for interacting with the DNA. Therefore, the reduction of metabolism and lack of mutagenicity associated with increased chlorination of the biphenyl ring is in direct opposition to the proposition that an arene oxide intermediate, capable of genotoxicity, is responsible for the tumorogenic effects of highly chlorinated biphenyls. It should be noted that phenanthrene, which is probably the PAH most closely related to biphenyl by structure, is not mutagenic in the Ames assay (McCann et al. 1975) and does not induce tumors in newborn mice, nor does the epoxide of phenanthrene (Grover et al. 1975). On the basis of structure activity, relationships of PAHs, it would seem more prudent to predict that PCBs would be more likely to act like the noncarcinogen phenanthrene than the carcinogen benzanthracene. More recently, it has also been suggested that the actual ultimate carcinogen among the PAHs is a dihydrodiol-epoxide metabolite which is much more mutagenic and carcinogenic than the parent compound (Kapitulnik et al. 1977). Since epoxide formation becomes less likely with increased chlorination of the biphenyl nucleus, it would seem that formation of dihydrodiol epoxide intermediates are un-

likely to correlate with either observed tumorogenicity Another feature of PCB metsbolism that should be considered when postulating potential mechanisms for cancer induction is the species differences in the rate of metabolism. Based upon the formation of reactive metabolites leading to tumor induction, it may be possible to induce cancer in rodents but much less likely to do so in primates or man. This stems from the finding that rats metabolize and eliminate 76% of a dose of PCBs in 3 days while primates eliminate only 2% of the same dosage for the same interval (Van Miller et al. 1975). Thus, not only would mutational events leading to cancer be less likely to occur in primates, but DNA repair mechanisms would also be much more likely to prevent the permanence of any mutational event.

4.2. Acute & Subchronic Toxicity. Several reviews of the mammalian toxicity of PCBs have appeared in the last decade (Fishbein 1974, Kimbrough 1974, Peakall 1975, NIOSH 1977, IARC 1978, EPA 1980). Therefore, an exhaustive review of the literature will not be offered here. A summary of the pertinent acute side effects of PCBs in animals follows.

LD_{50}'s are large for most species tested. In rats, the acute lethal dose ranges from 4,000 mg/kg - 16,000 mg/kg and increases with the chlorine content of the mixture tested (Fishbein 1974). The acute toxicity of PCBs may be classified as slightly toxic to non-toxic as described in Tables 5 and 6 (Doull et al. 1980).

Skin disorders similar to those seen in humans have been observed in monkeys, where facial edemas, hair loss and acne occur at oral doses of 250-400 mg (Allen et al. 1974). PCBs applied directly to the skin of rabbits induce hyperkeratosis, erethyma, blisters and desquamation (IARC 1978).

Endocrine effects are another change elicited by PCBs. Estrogenic activity, which is possibly related to effects on steroid metabolism, has been reported in rats (Higuchi 1976). In primates, PCB exposure results in prolonged menstrual cycles and increased bleeding (Barsotti et al., 1976).

Other symptoms reported in various species include: gastric hyperplasia, thymic atrophy, decreases in red blood cells and lymphocytes, splenic atrophy, and an increase in the serum level of triglycerides, cholesterol and phospholipids (for review see NIOSH 1977, IARC 1978, Higuchi 1976 and EPA 1980)

4.3. PCB Contaminants. No discussion of the toxicity of the polychlorinated biphenyls can be complete without stressing the possible role of trace contaminants of PCBs, e.g., the polychlorinated dibenzofurans. For example, embryotoxicity of the PCBs has been attributed to chlorinated dibenzofurans present as trace contaminants in the commercial preparations. Subsequently, terra-, penta-, and hexachlorodibenzofurans were detected in a number of American preparations of PCBs (e.g., Aroclor 1248, 1254, 1260). Concentrations of the individual polychlorodibenzofurans were in the order of 0.1 mg/kg of the PCB Chlorinated dibenzofurans have been considered as possible causes of embryonic mortality and birth defects observed in PCB-feeding experiments in birds. The polychlorinated dibenzofurans are structurally related to the chlorinated dibenzo-p-dioxin, some of which are highly toxic, teratogenic and carcinogenic.

A number of possibilities exist to account for the presence of polychlorodibenzofurans in commercial PCB mixtures. One explanation considers the presence of the parent compound (dibenzofuran) in the technical grade biphenyl subjected

to the chlorination process. It is also conceivable that polychlorinated dibenzofurans may be produced from PCBs in the environment.

It should be stressed that the transformation of only 0.002% of a major constituent of an Aroclor mixture to the corresponding chlorinated dibenzofurans would produce concentrations in the mixture corresponding to the values reported by Vos et al. (1970) as toxicologically significant.

5. MUTAGENICITY

The Ames assay utilizes Salmonella typhimurium to detect reverse point mutation at the histidine locus. Only monochlorobiphenyls have demonstrated any activity in the Ames 1538 tester strain. In this same study, the polychlorinated biphenyls such as 2, 2', 5, 5' tetrachlorobiphenyl, 1254 and 1260 were negative (Wyndham et al. 1976; Safe, EPA 1980).

Green et al. (1975a) have demonstrated that PCBs do not cause significant clastogenic effects in bone marrow or sperm cells of the rat even at high doses. Aroclor 1242 was given at a single dose of 1250 - 5000 mg/kg and at 500 mg/kg for four days (a regimen causing the condition of the animals to deteriorate), while Aroclor 1254 was given at doses of 75 - 300 mg/kg for five days. These findings are consistent with the lack of chromosomal aberrations observed in human lymphocyte cultures with doses of 100 mg/kg Aroclor 1254 (Koopingarner et al 1972).

The possible mutagenicity of PCBs has been studied by Green et al. (1975b), using the dominant lethal test. There was no statistically significant increase

in the number of dead implants, again at high dosages of Aroclor 1242 and 1254. Keplinger et al. (1971) also employed the dominant lethal assay and reported no evidence of mutagenic effects for the Aroclors. Polychlorinated biphenyls do not have significant mutagenic potential.

6. REPRODUCTION

Studies in various species indicate that PCBs do not affect conceptual rates in animals. Calandra (1976) studied the effects of Aroclor 1254 and 1260 in rats through three generations. In the first generation of offspring, he found no changes related to treatment in the mating index, in the second generation he found reduction, and in the third generation he found results similar to those of the first generation. Calandra concluded that there was no suggestion of any alteration in response in succeeding generations. Similarly, Linder et al (1974) found no effect on reproduction in Sherman strain rats exposed through two generations at dietary levels of 5 ppm. Exposure of rats to 50 mg/kg/day of Aroclor 1254 or 100 mg/kg/day of Aroclor 1260 also did not effect reproduction (Linder 1974). In the rabbit, Villeneuve et al. (1971) found no decrease in the number of pregnancies in animals fed 0.1 or 1.0 mg/kg body weight of either Aroclor 1221 or 1254. Finally, female rhesus monkeys given Aroclor 1248 at 2.5 or 5.0 ppm in their diet and bred to untreated males had normal conception rates (Barsotti et al. 1976).

Other indices of reproductive toxicity were similary unchanged by PCBs. Calandra (1976) noted that neither of the reproductive tracts of male nor female rats were affected following exposure to PCBs. Dikshith et al. (1975) reported that Aroclor 1254 produced no histopathological changes in the testes or epididymis,

nor did this Aroclor 1254 cause significant chromosomal damage or arrest spermatogenesis in the male rat. Animal studies indicate that there is little reproductive risk associated with exposure to PCBs, a finding which agrees with the lack of reported fertility problems in humans after exposure or consumption of PCBs.

7. BIRTH DEFECTS

Aroclor 1242, 1254, or 1260, at doses up to 30 mg/kg administered to rats during the organogenic period of gestation, did not produce excess embryotoxicity or morphological defects (Calandra 1976). Other experimental evidence documents the lack of adverse effects of PCBs on either embryo or fetal development. Linder et al., (1974) reported that administration of Aroclor 1254 to pregnant rats at 100 mg/kg/day on gestational days 7-15 did not produce any grossly abnormal offspring. Although only 30% of the offspring of mothers exposed to 100 mg/kg/day survived until weaning, greater than 20% of the treated mothers died prior to delivery. The 20% maternal mortality produced by the treatment suggests overt maternal toxicity contributes to neonatal mortality. The increase in liver weights noted among these offspring was sporadic and not dose related, which minimizes the likelihood that the increased liver weight reported was a specific PCB-induced effect. In other studies, Aroclor 1254 administered during gestation again resulted in no fetal morphological abnormalities or reduction in viability (Villeneuve et al. 1975). PCBs also did not induce terata in pups born of dams fed the equivalent of 12 ppm in the diet or in piglets of sows fed the equivalent of 30 ppm in the diet. However, increasing these consumptions by 4 to 30 times apparently results in some form of terata (NIOSH 1977). These data cannot be properly evaluated since they have not been published. Doses of PCBs up to 300 mg/kg were not teratogenic in mice when given on gestational days 1

through 6 or on gestational days 7 through 11 (Tocruek 1973). Similarly, feeding pregnant monkeys Aroclor 1248 at levels of 2.5, 5 or 25 ppm did not produce malformations (Allen et al. 1974, Barsotti 1976 and Allen and Barsotti 1976) However, because of overt maternal toxicity resulting from exposure to the higher levels, abortions were common, as well as reductions in fetal body weights PCBs have not significantly altered embryo or fetal development in either rodents or subhuman primates and there appears to be little teratogenic risk associated with exposure to PCBs.

8. CARCINOGENICITY

The polychorinated biphenyls (PCBs) present distinct problems when attempts are made to estimate a potential cancer risk to man from data developed in rodent studies. The crux of the dilemma is the need for a relevant interpretation of an excess incidence of liver tumors in rodents fed certain PCBs. Similar problems in interpreting hazard, based upon an excess of rodent liver tumors, have been experienced for a number of other structurally-related persistant, halogenated organic compounds. Included among these related compounds are DDT, dieldrin, mirex, Kepone®, hexachlorobenzene, polybrominated biphenyls, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and hexachlorodibenzodioxin. The polyhalogenated dibenzofurans, naphthalenes, and terphenyls which are related structurally, have not been tested for a carcinogenic effect. Most of the chemicals mentioned are of special concern, since they are persistent in mammals once they have been absorbed and poorly degraded in the environment. While most of the chemicals representing these classes are commercial products, the polyhalogenated dibenzodioxins and dibenzofurans occur only as contaminants of other products. The following expanded discussion of oncogenesis and carcinogenesis emphasizes the

comparative pathology of lesions produced in the liver of rodents by chronic PCB administration.

8.1. What Mechanisms Provide for the Expression of Carcinogenicity or Oncogenicity? For the purpose of our discussion and, as opposed to NCI suggestions for classification (which are discussed later), cancer will be defined traditionally. Cancer is the production of life threatening malignant tumors of potentially unlimited growth that expand locally by invasion and systemically by metastasis. A chemical is by definition only a carcinogen under those conditions which irreversibly initiate somatic mutations, promote uncontrolled neoplastic transformation and ultimately produce cancer. A chemical will be defined as a carcinogen only under limited circumstances. Several hormones and elements are essential at physiological levels and carcinogenic at toxic levels. A chemical is only a carcinogen under conditions which irreversibly initiate and ultimately produce cancer.

Initiation is defined as the production of damage to DNA which is of a magnitude sufficient to be sustained but of a nature which permits cell survival and predisposes the capacity of uncontrolled growth. Cells containing damaged DNA can, under certain circumstances, act as the stem cells for the formation of clones of cells containing heritable mutations. Mutagenicity is defined as the production of permanent alterations in the genome. The related processes of somatic mutagenesis and initiation have the potential to alter the genome of any cell. An altered genome inevitably increases the probability of dysfunction. Cancer is cellular regulatory dysfunction expressed as irreversible and uncontrolled growth. Heritable somatic mutations which result in the initiation of genes responsible for cellular regulation increase the probability of producing

cancer.

Most mammalian genes exist in allelic pairs. Evidence from somatic cell hybridization studies indicates that the malignant phenotype appears to be recessive for non-viral induced cancers. Consequently, a recessive somatic mutation in one of the alleles would not normally be expected to manifest itself as cancer until a second mutation occurred in the remaining normal allele carried in the same cell. The probability of two independent mutations occurring in the same allelic pair of genes in the same cell is extremely rare. However, given enough mutations in enough cells and a prior inheritance of susceptable genes through the germ line, the chance of having a cell with both alleles of a critical gene being mutated and thereby producing cellular dysfunction becomes not just probable but very likely. In the case where there is a history of a spontaneous incidence of cancer in the test animal, dysfunction is inevitable and the question changes from whether or not cancer will develop to how much and how soon cancer will develop. Liver cancer, which is very common in the rat and mouse, has been shown to be an example of this process. Cancer risk increases with time, primarily because the magnitude of initiation is a cumulative probability.

Rodent liver tumor causation is often referred to as multifactorial. This is just another way of saying many indirect factors can alter the incidence of tumors. Total calories, fat content, vitamin E, stress, crowding, and sex of the host can all influence tumor incidence. Therefore, in order to be specifically defined a carcinogen, rather than a factor of unknown mechanism, a compound must be an initiator. Initiation, the sustained modification of genes via specific alteration of somatic cell to DNA, is dose dependent. Thus, whether and when a chemical is or is not defined as a carcinogen is dependent on dose. How then do

we categorize compounds which increase the observed incidence of tumors when the data available describing the tumors are not sufficient to strictly or unambiguously classify the tumor as cancer?

Promoters have been postulated to act not as initiators but through modulation of gene expression and cellular communication. The molecular mechanisms of promotors are more varied than those of initiators which must alter DNA. Included among the known mechanisms of promotion is the ability to control various genes which provide a selective growth advantage. We will define promotion as the ability to enhance the selective proliferation of previously initiated cells. By definition promotion cannot occur until a critical gene is initiated. The DNA of dividing cells replicates proportionately to the rate of cell division. DNA is more susceptible to mutation by chemicals during replication. Silent mutations of dormant cells are more likely to be expressed during replication. Wounding, growth stimuli, necrosis, inflammation, or certain chemicals can selectively and rapidly allow cells containing the mutated gene to form a clone of semi-initiated cells. A rapidly dividing clone of initiated cells containing one mutation of a critical gene obviously enhances the probability per unit time of initiating a second mutation of the remaining normal allele. "Foci" or "Areas" discussed in detail later are likely clones of cells rapidly developing in response to chronic injury inflicted by PCB treatment.

How can we account for progression as well as regression of tumors? The biological environment controls normal growth, development, and maturation. These controls constrain the growth of cryptic in situ cancer. When these controls deteriorate, as in old age, or are overwhelmed by external influences, cancer develops. The growth of most cells with malignant phenotype in healthy animals

MONS 016061

is repressed by contact with normal cells through a process called contact inhibition. Small molecules and macromolecules responsible for contact inhibition, including membrane antigens, are readily transferred between cells. Therefore, even if a mutation, reducing critical gene products controlling differentiation and growth, occurred in one gene of a cell, the close physical presence (contact inhibition) of normal cells would contribute to maintaining the required level of gene products. The net effect is that healthy tissue can usually repress the tendency of initiated cells to proliferate. When initiated cells do proliferate, cells in the center of the forming clone will be subjected to less and less contact with normal cells. Less control increases the opportunity for the clone to further undergo differentiation or incorporate a second mutation during the period of proliferative activity. After a homozygous mutant is formed, the likelihood of either a normal homozygous or heterozygous cell being able to transfer enough of the needed gene products even by direct contact is greatly reduced. Escape from the anti-proliferative influence of normal cells is also enhanced by the establishment of a critical mass of mutated cells; such a critical mass also aids the clone in evading immune surveillance.

Our hypothesis is based on the basic assumptions that each somatic cell of the differentiated mammalian organism (rat, mouse or man) contains the same genes; each cell type has only a few specific genes in the active state; each differentiated cell type has specific gene products; and the various differentiated cells of the organism contain receptors which differentially respond to specific sets of environmental signals. If we assume that molecular signals detected by the membrane can all be transferred to the nucleus and then to specific genes, a mechanism by which a multi-differentiated organism can specifically respond and adapt to the environment has been constructed. Receptors of various cell types

are not likely to be equally influenced by any chemical since they are coded by different genes. Therefore, it is only reasonable that there are tissues where the production of mutations may or may not be expressed as dysfunction.

Gene dosage is a related phenomenon whereby the effective level of gene products is determined by phenotype. The quality as well as the quantity of selected gene products influence the maintenance of the non-proliferative state via messengers for the receptors producing cell-division repression. Cell proliferation will occur if cells producing cell-division repressor messengers are destroyed, modified or disrupted by chemicals, surgery or wounding; if the ability of repressors to act is inhibited; or if the receptor type or numbers have been altered by mutational or epigenetic repression. If the damage is reversible, cells may retain the ability to restore the original number, levels, and state of gene products. Under such conditions, the cell is capable of reestablishing feedback control over proliferation/differentiation.

Any event facilitating a second mutation of a gene resulting in a cell type homozygous for the neoplastic phenotype will facilitate eventual progression of the resulting cell line into a disease state expressed as malignant, true breeding, metastasizing tumors. Agents that only promote the ability of preexisting phenotypes to be expressed would be expected to accelerate the rate of development but not necessarily create a new disease type. Therefore, appropriate diagnosis of cancer would be accomplished by making comparisons of malignancy only at points in time when spontaneous lesions in the controls and induced lesions in the treated occur at the same incidence. Processes only promoting cancer in situ would be expected to regress upon removal of the promoter and not change the ratio of metastasizing to nonmetastasizing tumors. It is noteworthy that

such often appears to be the case with liver cancer in rodents produced by cyclic chlorinated compounds.

A compound can increase cancer either by initiation or selective proliferation of clone(s) of naturally occurring neoplastic cells or by a combination of both processes. Tissue compound interaction characteristics mediate the expression of these basic processes and ultimately the presence or absence of tumor development. A compound is an incomplete carcinogen when it only initiates or only promotes. For example, when the level of administration of an initiator is not sufficient to kill any or many cells and a hyperplastic response is not produced, other sources of promotion are needed to amplify the growth characteristics of initiated cells. If, on the other hand, the exposure to an initiator kills or injures many of the target cells, the rapid growth of progeny of the surviving initiated cells will be triggered by the process of repopulating the organ. In this case the initiator, through its cytotoxic action, is both direct initiator and promoter and, therefore, a complete carcinogen. If a compound is present at a level which promotes selective growth of cells with neoplastic phenotypes but does not sustain compound induced mutations, the compound would be functioning only as a promoter. Since both promotion and initiation are independently dose- and time-dependent and tissue, sex, and species sensitivities vary, the definition of a compound as an initiator or promotor is dose, duration, and route, as well as tissue, sex, species, and time-dependent.

It will be documented later that PCBs do not increase the incidence of tumors at any site except the rodent liver. Liver cell death, trauma and hyperplasia are all produced by PCBs and can be expected to yield conditions favoring preferential growth of cells with dormant neoplastic phenotypes. Liver tumors are a

common part of the natural disease process of rodents One can safely generalize that the tumorigenic properties of PCBs to the rodent liver result at least in greatest part from alteration of the homeostasis of control mechanisms Thus, changes seen in rodents with a natural history of high incidence of liver tumors must be interpreted carefully. Toxic response and promotion must not be mistaken for initiation.

8.2. Comparison of the Characteristics of Oncogens and Effects of PCBs

1. Oncogens produce increases of spontaneous tumor at selected sites. PCBs produce liver tumors.
2. Tumors produced by oncogens usually do not increase the metastatic characteristics of spontaneous tumors of the same site. PCB increased liver tumors do not metastasize.
3. Oncogens do not produce transplantable tumors unless the naturally occurring tumor is capable of transplantation. PCB tumors do not transplant.
4. Tumors influenced by oncogens are often affected by factors such as nutrition, stress, chronic injury, and sex of the animal. Liver tumors are affected by these factors.
5. Oncogens need not be mutagenic. PCBs are not mutagenic.
6. Neoplastic lesions increased by oncogens may be reversible. PCB increased liver tumors regress.
7. Progression of differentiation of neoplastic lesions increased by oncogens often ceases when stimulus is removed. Continued presence of PCBs is required to sustain liver neoplasia.
8. Neoplastic effects of oncogens are usually associated with the chronic dysfunction of the effected site. PCBs produce chronic hepatotoxic responses.

MONS 016065

9. Oncogens do not necessarily increase the effect of carcinogens acting at the target site. PCBs reduce the response of several other liver carcinogens

10. Oncogens affecting the liver often increase microsomal enzyme activity and eventually produce evidence of metabolic dysfunction. PCBs stimulate SER and produce metabolic dysfunction.

11. Oncogens rarely if ever increase tumor incidence at exposure levels producing no toxic effects. PCBs are tumorogens only at toxic doses.

12. Oncogenic effects often require continued presence of the stimulus. Continued presence of PCB is required to sustain neoplastic alterations of the liver.

8.3. Comparison of the Characteristics of Carcinogens and the Effects of PCBs

1. Carcinogens usually produce neoplastic lesions at multiple sites. PCBs only increase lesions of the liver.

2. Carcinogens often increase the malignancy of spontaneous tumors at the same site. PCB increased liver tumors are of low malignancy.

3. Carcinomas irreversebly progress once a critical tissue mass is established. PCB increased tumors can regress.

4. Carcinogens produce tumors which metastasize. PCB increased liver tumors do not metastasise.

5. Carcinogens produce lethal tumors. PCB increased liver tumors are not lethal.

6. Carcinogens produce transplantable tumors. PCB increased liver tumors do not transplant.

7. Carcinogens are often effective at single exposures. PCBs increase liver tumors only after prolonged continuous exposures.

8. Carcinogens are often active at nontoxic doses. PCBs only increase liver tumors at hepatotoxic doses.
9. Carcinogens are initiators. PCBs are not initiators and therefore not carcinogens.
10. Initiators are mutagens. PCBs are not mutagens and therefore not initiators.

It is apparent that the neoplastic effects of PCBs match the definitions of an oncogen and do not match the definitions of carcinogens.

8.4. What Has Been Characterized as Cancer in The Bioassay Literature? There has been great controversy over the relevance and nomenclature of rodent hepatic lesions. Most of the controversy surrounds the uncertainty associated with the diagnosis of cancer histologically prior to progression to the classical malignant state.

The National Cancer Institute sponsored a workshop on the classification of hepatocellular tumors and related lesions of rats. There were 20 participants. A recommended classification and nomenclature of the liver lesions resulted (Squire and Levit 1975). These recommendations were not unanimous and have not been universally accepted, but are utilized in many of the government supported reports of PCB carcinogenesis. The following definitions reflect the NCI position. Comments have been added when relevant to emphasize the general effect of the NCI recommendations that can be characterized as enforcing an expansive philosophy in defining the neoplastic potential of liver lesions.

8.5. Cholangiofibrosis (Adenofibrosis). This lesion is characterized by foci or areas of hyperbasophilic, atypical ducts in a fibrous stroma. In most cases, there later develops an excessive formation of collagen or of cystic glandular spaces. These lesions could be the "clones" referred to earlier. The nature of the lesion is controversial but was not considered precarcinogenic by Stewart and Snell (1957).

Adenofibrosis referred to by Kimbrough is identified as the cholangiofibrosis referred to by Ito. Kimbrough and Ito have observed adenofibrosis in rats and mice that were fed PCBs. The PCBs tested by Kimbrough, producing adenofibrosis, were contaminated with trace amounts of chlorinated dibenzofurans. Adenofibrosis can but does not always occur concomitantly with hepatocellular carcinoma in rodents. Although increased incidence of hepatocellular tumors have also been reported in rodents after exposure to DDT, Dieldrin, Mirex, and Kepone, these compounds do not produce adenofibrosis liver pathology. Kimbrough speculated that it is possible the PCBs cause adenofibrosis through lipid peroxidation, while Mirex, Kepone, DDT, and Dieldrin do not.

8.6. Foci or Areas of Cellular Alteration. The choice of the term "foci" versus "area" has traditionally depended upon the judgement of the pathologist. The term "foci" is used for small lesions less than 1 liver lobule in size. The term "area" was recommended by NCI for designating lesions approximately as large as or larger than a lobule. The primary alterations involve the tinctorial qualities and textural appearance of the cytoplasm of hepatocytes, and the recommended terms are purely descriptive. There is no obvious disruption of the liver architecture, and the plates of affected cells merge without demarcation with surrounding liver tissue. Affected liver cells may be larger or smaller

than normal hepatocytes, and some nuclei may be enlarged, vesicular, or hyperchromatic and have large nucleoli. The cells in ground glass or eosinophilic foci are usually enlarged due to an increase in cytoplasm. The cells in basophilic foci have a diffuse cytoplasmic basophilia and may be larger or smaller than normal liver cells. Clear cells are usually normal in size or somewhat larger

The nature of these lesions is controversial. Some feel that the basophilic "foci" or "areas" have greater significance with respect to tumor development than do the other cellular alterations. Most agree that "foci" or "areas" are cytologically similar to the cellular elements of neoplastic nodules and some feel that "foci" or "areas" may possibly be part of the spectrum capable of progressing to the formation of nodules. These lesions must be considered to be functionally equivalent to clones.

8.7. Neoplastic Nodules. This term was suggested by NCI to replace "hyperplastic nodules." The term describes spherical lesions that usually occupy an area equivalent in size to that of several liver lobules in which the normal liver architecture is absent within the nodules. Hepatocytes within the nodules are similar to those in foci or areas and may show mixtures of the cytoplasmic alterations. Mitoses and varying degrees of nuclear atypia including enlargement, hyperchromasia, doubling in number, and enlarged nucleoli are sometimes present. The cells may be arranged in solid or jumbled sheets or in irregular plates, one or more cells thick. Sinusoids may be compressed by enlarged hepatocytes or show varying degrees of dilation or ectasia. Portal areas are usually not present, although in rare cases they may be localized inside the nodules. An important feature is the architectural distortion and sharp demarcation of the nodule from the surrounding liver around at least a portion of its

periphery. The plates of nodules' cells are usually not continuous with those of unaffected liver; rather they impinge perpendicularly or obliquely upon the tangentially arranged normal plates. The latter are often narrowed due to compression by the expanding nodule.

The NCI decisions to recommend the term neoplastic nodule was based upon the NCI conclusion, not universally shared, that the experimental and biological evidence available justified it. The NCI evidence is interpreted as suggesting that such nodules are proliferative lesions, and few would argue this point; however, NCI goes one step farther and states that nodules are known to be induced by carcinogens and, at the least, nodules indicate an increased probability for the development of hepatocellular carcinoma. Most workers would agree that chronic injury will result in proliferative lesions but most workers would also agree that, at least initially, these lesions have the capacity to regress upon removal of the agent causing injury. Chlorinated compounds present unique problems because their persistance complicates experiments studying removal of the agent. It should be pointed out, however, that these lesions are typical of other compounds such as phenobarbital. The difference is that phenobarbital is rapidly excreted. The induction of proliferative lesions due to chronic cellular injury is part of a predictable biological response and should not be confused with similar lesions that are associated with the irreversible process of carcinogenesis.

Neoplastic nodules are considered by NCI and others to represent part of the spectrum of response elicited by hepatocarcinogens in rodents. However, this view is not shared by all scientists in the field. Persons subscribing to this thesis suggest that areas of alteration will develop, that some of these will

become nodules and that some of the nodules will in time transform into hepatocellular carcinomas. A number of carcinogens have produced this spectrum of response. It is cautioned that there need be no difference in the observed response spectrum even when the chemical producing the nodule is an unambiguous genotoxic carcinogen or one of the polyhalogenated cyclic hydrocarbons.

8.8. Hepatocellular Carcinoma. The diagnosis of hepatocellular carcinoma by NCI is based upon characteristic histological and cytological features whose correlation with cancer NCI claims is well documented in the pathology literature. This definition eliminated the necessity of observing several classic endpoints including invasion, metastasis and lethality.

Hepatocellular carcinomas, by NCI definition, are usually considerably larger and more irregular than neoplastic nodules, and they may involve major portions of liver lobes At the periphery, they compress or extend into the surrounding parenchyma. Trabecular carcinomas may be classified as well to poorly differentiated, depending upon their resemblance to normal liver. Tumor cells are in broad sheets or in plates one to several cells in thickness. The latter are haphazardly arranged in linear, papillary or pseudoacinar patterns. Tumor cells may also be individualized or in isolated nests and cords enveloped by lining cells. A histological variant of hepatocellular carcinoma is the carcinoma with a predominantly glandular, papillary pattern, resembling adenocarcinoma.

According to NCI, much variability in lesion architecture is permissible. For example, the tumor cells may resemble normal hepatocytes, or they may be enlarged or anaplastic in less well differentiated tumors. The cytoplasm may be clear, eosinophilic or hyperbasophilic, and nuclei are frequently enlarged and

MONS 016071

hyperchromatic. Multiple nuclei and mitotic figures may be present. This excessive inclusiveness and lack of strict minimal criteria were necessary if lesions produced by polyhalogenated cyclic hydrocarbons were to be consistantly diagnosed as carcinoma.

It was concluded by NCI that benign hepatic cell tumors, i.e., without potential for malignant behavior, could not be consistantly diagnosed. Therefore, terms such as "adenoma" were not recommended. It was also agreed that the term "hepatoma" was imprecise in its usage and was not recommended for any of the lesions under discussion at the workshop.

The recommendations developed by NCI allow for the inclusion of proliferative lesions that are clearly capable of regressing when the stimulus is removed. Detection of vascular invasion or metastases in contrast to tradition was not considered by NCI to be essential for the diagnosis of hepatocellular carcinoma. The utilization of inclusive cytological criteria to define what might have the potential to become the disease process we call cancer was accomplished at the expense of including many responses which will never progress, invade, metastasize or kill.

8.9. What Level of Confidence Can be Placed on Liver Tumors? Over the past several years, 54 or 23% of the 230 chemicals tested by the National Cancer Institute (NCI) have been found to induce hepatocellular neoplasms in rats and/or mice. What predictive power do these results hold for estimating risk to the human disease CANCER?

If we are to make rational decisions, we must appreciate the comparative patho-

MONS 016072

genesis of rodent liver neoplasia. The hepatocellular neoplasms of mice have variously been referred to as liver tumors, hyperplastic nodules, type A or B nodules, and hepatocellular adenomas and carcinomas (Ward and Vlahakis 1978, Frith and Ward 1979, Butler and Newberne 1975). The biological behavior of these nodules has been and continues to be a subject of heated debate.

It has been demonstrated, at least in some systems, that some of the poorly differentiated small nodules can grow progressively to larger nodules that are transplantable and metastasize to other tissues (Ward and Vlahakis 1978, Williams et al. 1979). The small and/or better differentiated nodules (sometimes called hyperplastic nodules, adenomas, or type A nodules) are usually not transplantable and do not metastasize (Butler and Newberne 1975). The morphology and biologic behavior of these nodules are similar to those of adenomas in other murine tissues (Ward and Vlahakis 1978). However, foci of trabecular carcinoma often appear in these adenomas (Frith and Ward 1979, Ward and Vlahakis 1978).

The larger and/or less differentiated nodules (termed hepatocellular carcinomas or type B nodules) usually appear morphologically different from small nodules (Frith and Ward 1979). A few carcinogens induce hepatocellular neoplasms that appear the same from the time they are small tumors (early stages) until they become metastatic tumors (Butler and Newberne 1975, Ward et al. 1979). Some lesions have been classified by certain authors as hyperplastic nodules without evidence that they were not neoplastic or did not represent the early stages of carcinoma.

There are reports (Ito et al. 1976, Periano et al. 1973) that indicate that he-

MONS 016073

patocellular nodules in mouse liver may regress if exposure to the alleged carcinogen is discontinued at a specified time. Studies with other carcinogens demonstrate this does not always occur (Frith and Ward 1979, Butler and Newberne 1975).

An extreme view suggests that all hepatocellular neoplasms originate as carcinomas (Stewart 1975); therefore, chemicals inducing these tumors are carcinogens, even if the carcinomas do not invade or metastasize. A more moderate view is that some, but not all, liver tumors termed adenomas, hyperplastic nodules or type A nodules may represent an early stage of carcinoma formation (Ward and Vlahakis 1978). When evaluating the following oncogenicity studies, the reader is cautioned that the meaning given certain terminology is that of the original author's and no attempt has been made to provide translations.

8.10. PCB Oncogenicity and Related Studies. The polychorinated biphenyls (PCBs) present distinct problems when attempts are made to estimate a potential cancer risk to man. The crux of the dilemma is the need to develop a relevant interpretation of cancer risk from reports of an excess incidence of rodent liver tumors of unknown etiology. The effects covered in the following expanded discussion of liver toxicity emphasize lesions produced by PCBs in the liver of rodents.

8.10.1. General Hepatotoxic Effects. Polychlorinated biphenyls induce microsomal mixed-function oxidases and cause hepatomegaly in rodents and other mammals. Hepatomegaly has been interpreted by Kimbrough to be the result of the hypertrophy of individual hepatocytes. Hyperplasia also commonly occurs and increased mitotic activ-

ity can occasionally be noted. Hepatocytes enlarge and may accumulate lipid in their cytoplasm. At the ultrastructural level, enlarged hepatocytes show an increase in smooth endoplasmic reticulum and inclusions within the cytoplasm, which appear like concentric whorls, surrounding lipid vacuoles. Morphologic changes in the mitochondria have also been described (Kimbrough et al. 1972). In addition to these alterations, PCBs induce experimental hepatic porphyria (Goldstein et al. 1974). In the rat, experimental hepatic porphyria only occurs in the Sherman strain female. This observation indicates a unique sensitivity for the Sherman female rat. On microscopic examination, an increase in macrophages and prominent Kupffer cells containing brown ceroid pigment and necrobiosis of liver cells is prominent. Lipid accumulates in the cytoplasms of hepatocytes, resulting at times in hepatocytes with foamy cytoplasm.

Acute as well as chronic toxicity of PCBs has been studied in rats, monkeys, mice and cows (DHEW 1976, Kimbrough, et al. 1972, Allen, et al. 1976) and the organ consistently affected was the liver. For example, when male Sprague-Dawley rats were fed a diet containing mixtures of PCB isomers (Aroclor 1248, 1254 and 1262) at a concentration of 100 ppm in the diet for 52 weeks, there was an increase in their serum lipids and cholesterol and a transient increase in triglycerides accompanied by distinct morphological changes in the liver (Allen et al. 1976). Generalized liver hypertrophy and focal areas of hepatocellular degeneration were followed by a wide spectrum of repair processes. The tissue levels of PCB were greater in the animal receiving

the high chlorine mixtures and high levels persisted after the PCB treatment had been discontinued.

8.10.2. Neoplastic Effects. Nishizumi, (1970) studied the effects on mouse and monkey liver of long-term oral administration of polychlorobiphenyls (PCB), 1.5 mg/day or more, at selected intervals by light and electron microscopy. Hepatocytes of treated mice contained large amounts of acidophilic materials in the cytoplasm, and fatty vacuoles were observed later. Structural changes in the hepatocytes consisted of a marked increase of smooth endoplasmic reticulum, a reduction of rough endoplasmic reticulum, "myelin figure" formation in the cytoplasm, and an increase of microbodies and lysosomes. A marked increase in lipid droplets was observed later. Results of electron microscopy evaluation of monkey liver showed an increase of smooth endoplasmic reticulum in the hepatocytes and swelling in the Kupffer cells, with an increased number of lysosomes and vacuoles. Judging from the findings by electron microscopy, characteristic lesions of liver cells were produced by PCB administration. Kimura and Baba. (1973) observed similar changes in the liver of rats. Grossly, all the rats ingesting more than 700 mg of PCB showed hypertrophy of the liver. Pinhead- to pear-sized (sic.) round and pale brown flecks or nodules were scattered on the surface and on the cut-surface of the liver of all the female rats in the experimental group ingesting more than 1,200 mg of Kanechlor-400. None of the male rats showed such visible nodular changes in the liver in spite of having ingested a corresponding or even higher amount of PCB than females. Again a female rat selective sensitivity is observed.

Microscopically, changes in the liver of experimental rats showed fatty degeneration and multiple adenomatous nodules. The former was seen, irrespective of sex, in all the animals of the experimental group, but only in 2 females in the control group. The latter, which appeared to be a benign neoplastic lesion, was seen in all the female rats ingesting more than 1,200 mg of Kanechlor-400 as predicted by the gross examination. In sharp contrast, however, the liver specimens of the male rats revealed no such nodular changes, even in the animals who had ingested a comparable or higher amount of Kanechlor-400 than females.

Lung abscesses, pneumonia, spleen atrophy, and intracranial abscesses were found frequently in the experimental group, and this suggested that the resistance of the rats treated with Kanechlor-400 to infection was lowered. Depilation also appeared frequently in the experimental group, especially in the females when the PCB intake amounted to about 600 mg. PCBs induced benign adenomatous nodules exclusively in female rats.

Nagasaki et al., (1972) studied the hepatocarcinogenic effects of polychlorinated biphenyls in dd mice. Strain dd mice with an average weight of 19.0g were used. A total of 114 mice were divided into the following 10 groups and the animals were fed on a basal diet (Oriental MBF) supplemented with various kinds of polychlorinated biphenyls, Kanecrol-500, Kanecrol-400, and Kanecrol-300, which are classified by the number of chlorines, purchased from Kanegafuchi Chem. Co., Osaka. The groups were as follows: Group 1, 500 ppm Kanecrol-500; Group 2,

MONS 016077

250 ppm Kanecrol-500; Group 3, 100 ppm Kanecrol-500; Group 4, 500 ppm Kanecrol-400; Group 5, 250 ppm Kanecrol-400; Group 6, 100 ppm Kanecrol-400; Group 7, 500 ppm Kanecrol-300; Group 8, 250 ppm Kanecrol-300; Group 9, 100 ppm Kanecrol-300, Group 10, basal diet alone. Each group contained 6 to 12 mice. The animals were given water and the experimental diet freely. After 32 weeks, mice were sacrificed with ether and examined histologically.

Grossly, 7 of the 12 mice (58.3%) in Group 1 had many tumors in the liver. The liver increased in weight, and had a rough surface with multiple tumors up to 0.2 to 1.0 cm in diameter. In other groups, except in Groups 1 to 3, no remarkable changes were observed in the liver of mice. Microscopically, hepatomas were observed in the liver of mice in Group 1. Some area of nodules showed an adenomatous pattern. Many necrotic foci were seen in Group 1 animals. Nuclear irregularities and mitotic figures were frequently seen in nontumorous areas of the liver in Group 1. However, microscopical changes in the liver were not observed in Groups 4 to 9.

The hepatomas induced in mice by the Kanecrol-500 of polychlorinated biphenyls appeared similar to those induced by the α-isomer of benzene hexachloride. Kanecrol-400 and Kanecrol-300 had no carcinogenic activity in the liver of mice.

Kimbrough, et al. (1972) fed male and female Sherman strain rats polychlorinated biphenyls Aroclor 1260 and Aroclor 1254 at 0, 20, 100, 500 and 1,000 ppm in their diet. Rats received the dietary levels for

eight months. Light microscopic changes consisted of hypertrophy of the liver cells, inclusions in the cytoplasm, brown pigment in Kupffer cells, lipid accumulation, and, at the higher dietary levels, adenofibrosis. Ultrastructural changes in the livers of exposed animals consisted of an increase in smooth endoplasmic reticulum and atypical mitochondria. Lipid vacuoles were occassionally surrounded by concentric membranes.

The epithelial component of adenofibrosis consisted of goblet cells and cells that resembled the epithelium that lines the bile ducts. In general, the effect of Aroclor 1254 on the liver was more pronounced than that of Aroclor 1260.

Allen and Abrahamson (1973) fed rats diets containing 0.1% of three polychlorinated biphenyls (PCBs) (Aroclor 1248, Aroclor 1254, Aroclor 1262) for six weeks; these rats showed a progressive enlargement of the liver. This liver hypertrophy is attributed to proliferation of the smooth endoplasmic reticulum, development of large membranous concentric arrays, and increase in lipid droplets within the cytoplasm of the affected liver cells. Liver homogenates had increased levels of protein and RNA and reduced concentrations of DNA, while the microsomal fraction had increased levels of protein and phospholipids, and reduced levels of cholesterol. Also, there were modifications in the activity of certain hepatic microsomal enzymes. By the sixth week, the animals progressed from a stimulatory effect on the liver by PCBs to a stage where regressive hepatic changes were occurring, such as a decreased activity of microsomal enzymes, dissolution of concen-

tric membrane arrays, vesiculation of the endoplasmic reticulum, and accumulation of lipid droplets within the cytoplasm of the affected cells.

Ito et al. (1973) studied the effects of technical grade polychlorinated biphenyls (PCBs) on mouse liver histologically and ultrastructurally. Pathologic studies were also made on the effects of PCBs on tumorigenesis induced by benzene hexachloride (BHC) in mouse liver. Neoplastic changes were observed in livers of mice fed a basal diet containing 500 ppm of the PCB, Kanechlor 500, for 32 weeks. Amyloid degeneration in the liver of mice was observed in groups fed a diet containing lower concentrations of PCBs. Histologically and ultrastructurally, the neoplastic areas of mouse liver induced by PCBs appeared to be typical of nodular hyperplasias and well-differentiated hepatocellular carcinomas induced by BHC. The effects of PCBs on neoplastic changes induced by isomers of BHC in the liver of mice fed a diet containing BHC with or without PCBs was determined. Only those receiving 100 or 50 ppm of the α-isomer developed nodular hyperplasia and hepatocellular carcinoma. However, among the groups fed BHC plus PCBs, only those receiving 100 or 50 ppm of α-BHC or 250 or 100 ppm of β-BHC developed nodular hyperplasia and hepatocellular carcinoma. Groups fed γ-BHC with or without PCBs did not show neoplastic changes of the liver. PCBs themselves increased hepatic neoplasms in mice and also added to the increase in tumors induced by α-BHC and β-BHC.

Burse, et al. (1974) fed rats 100 ppm of Aroclor 1242 (6.6 to 3.89

mg/kg/day) or 100 ppm Aroclor 1016 (6.9 to 3.5 mg/kg/day). Plasma, kidneys, urine, brain, liver, and adipose tissue were analyzed for polychlorinated biphenyl (PCB) residues at 0.5, 1, 2, 4, 6, 8, and 10 months of exposure. In additional groups fed the experimental diet for 6 months, PCB tissue levels were determined 2, 4, 5, and 6 months after the exposure to PCBs was discontinued. PCBs were highest in adipose tissue where a steady state was approached in 2 and reached in 4 months. Little PCB-derived material was excreted in urine.

After discontinuing PCB exposure, Aroclor 1016 was eliminated more rapidly from the organ than Aroclor 1242. Measurable residue levels were still present after 5 (Aroclor 1016) and 6 (Aroclor 1242) months of recovery. Microscopic examination of the liver showed enlarged liver cells with vacuolated cytoplasm and inclusions. Kimbrough and Linder (1974) fed two groups of 50 BALB/cJ inbred male mice 300 ppm of a polychlorinated biphenyl, Aroclor 1254, in the diet for 11 and 8 months, respectively. The 6 months' feeding was followed by 3 months' recovery. Two additional groups of 50 mice each were fed plain chow. All 22 surviving mice fed Aroclor 1254 for 11 months had greatly enlarged livers representing 25% of their body weight, whereas those fed the experimental diet for 6 months only had slightly, but significantly, enlarged livers. Adenofibrosis was observed in all 22 livers of mice fed Aroclor 1254 for 11 months but not in the other groups. Of the 22 mice fed 300 ppm Aroclor 1254 for 11 months, 10 had hepatomas measuring 0.1-1.5 cm in diameter. One of 24 surviving mice fed Aroclor 1254 for only 6 months, followed by a control diet for 3 months, had a hepatoma 0.3 cm in diameter. No controls had hepatomas

Ito, et al., (1974) observed liver weight increases in groups on diets supplemented with Kanechlor-500, -400, or -300, the increase being greatest in the group that received 1,000 ppm of Kanechlor-500 In several groups, irregular-shaped yellowish nodules of up to 1.0 cm in diameter were seen on the liver, but no cirrhotic changes were detected.

No hepatocellular carcinoma was seen in any of the rats, but areas of cholangiofibrosis were found in the liver of rats given 1,000 ppm of Kanechlor-500, -400, or -300. Nodular hyperplasia was found in 38 - 12.0% of the animals given Kanechlor-500, 30 - 12.5% in those treated with Kanechlor-400, and 6 - 4.3% of those which received Kanechlor-300. The incidence of nodular hyperplasia of the liver was highest in the groups given Kanechlor-500 and lowest in those given Kanechlor-300. Oval cell proliferation and bile duct proliferation were seen in all groups of animals treated with Kanechlor. Hypertrophic changes of liver parenchymal cells in centrilobular areas were clear in groups that received 1,000 ppm of Kanechlor-500 or -400. Amyloid degeneration of the liver was seen only in the group given 100 ppm of Kanechlor-400. No remarkable changes were seen in other organs of either experimental or control rats.

Makiura, et al., (1974) Studied the effects of polychlorinated biphenyls (PCBs) on liver carcinogenesis in rats treated with the hepatic carcinogens 3'methyl-4-dimethylaminoazobenzene (3'-Me-DAB), N-2-fluorenylacetamide (2-FAA), and/or diethylnitrosamine (DEN). Animals were examined histopathologically after they had received the exp-

erimental diet for 20 weeks and then the stock diet for 4 weeks. Liver tumors developed in 65.2, 33.8, and 92.3% of rats in groups treated with 0.03% 3'Me-DAB, 0.015% 2-FAA, and 0 0025% DEN, respectively. Rats that received 0.05% PCB only did not develop liver tumors, and those treated with PCB and the carcinogens developed only a few tumors. Multiple liver tumors developed after treatment with 0.03% 3-Me-DAB plus 0.0025% DEN (92.3% incidence) and 0.015% 2-FAA plus 0.0025% DEN (81.8%); after treatment with these combinations plus PCB, the incidence of liver tumors was very low or zero. Histologic examination showed that PCB inhibited development of nodular hyperplasias, oval cell infiltration, and bile duct proliferation as well as hepatocellular carcinoma induced in the liver by the chemical carcinogens.

Ito et al. (1974) induced nodular hyperplasias but not hepatocellular carcinomas in rats with PCBs. Some of the findings confirmed those of Kimura and Baba (1973). These studies showed that all kinds of Kanechlor are tumorigenic in the rat liver. Marked cholangiofibrosis (adenofibrosis) was seen in the rat liver.

Kimbrough et al., (1975) utilized Sherman strain female rats (200) fed 100 ppm of a polychlorinated biphenyl (Aroclor 1260) for approximately 21 months, and kept 200 female rats as controls. The rats were killed when 23 months old. Twenty-six of 184 experimental animals and one of 173 controls had lesions interpreted by histologic criteria as hepatocellular carcinomas. None of the controls but 144 of 184 experimental rats had neoplastic nodules in their livers. Areas of hepato-

cellular alteration were noted in 28 of 173 controls and 182 of 184 experimental animals. Thus the polychlorinated biphenyl Aroclor 1260, when fed in the diet, was interpreted as having a hepatocarcinogenic effect in these female rats. It is worthy of note that hepatocellular carinoma was based on the controversial NCI definition. The incidence of tumors in other organs did not differ appreciably between the experimental and control groups. Hepatocellular alterations were observed in a high percentage of the control animals.

Nat. Cancer Inst., DHEW Publication No. (NIH) 78-838 (1978) reported the bioassay of Aroclor 1254 for possible carcinogenicity. The bioassay was conducted by administering the test chemical in feed to Fischer 344 rats. Groups of 24 rats of each sex were administered Aroclor 1254 at one of three doses, either 25, 50, or 100 ppm, for 104-105 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks. Mean body weights of males and females receiving mid and high doses and females receiving low doses of the chemical were consistently below those of the corresponding controls, beginning at about week 10 of the study. The decrease in survival among males, but not among females, showed a significant dose-related trend. Adequate numbers of animals of both sexes survived for meaningful statistical analyses of the incidences of tumors.

The combined incidences of lymphomas and leukemias showed a significant dose-related trend in males (controls 3/24, low-dose 2/24, mid-dose 3/24, high-dose 9/24, P = 0.009). However, the direct com-

parisons of each dosed group with those of the matched controls were not statistically significant, and the tumors cannot clearly be related to administration of Aroclor 1254.

Hepatocellular adenomas and carcinomas were found in the dosed groups, but not in the controls (males: mid-dose 1/24, high-dose 3/24; females: mid-dose 1/24, high-dose 2/24). Additionally, a high incidence of nonneoplastic hyperplastic nodules was noted in the dosed animals (males: controls 0/24, low-dose 5/24, mid-dose 8/24, high-dose 12/24; females: controls 0/23, low-dose 6/24, mid-dose 9/22, high-dose 17/23). Although the incidences of tumors were not significant, the occurrence of the hyperplastic nodules appeared to be related to administration of the chemical.

In the stomach, jejunum, or cecum, adenocarcinomas were observed in two dosed males and in two dosed females as well as a carcinoma in one dosed male. None of these lesions was found in control animals in this study. Historical incidences of these tumors at this laboratory (6/600 males [1%], 2/600 females [0.3%] suggest that the lesions, although not statistically significant, may be related to the administration of Aroclor 1254. It was concluded that under the conditions of this bioassay, Aroclor 1254 was not carcinogenic in Fischer 344 rats.

8.11. Tumor Initiation Modification. The effects of PCBs on carcinogenicity of various chemicals have been investigated by numerous groups. Kanechlor-500 in combination with 3'methyl-4-dimethylaminoazobenzene, N-2-fluorenylacetamide

and diethylnitrosamine in the diets of rats markedly decreased the formation of hepatocarcinomas (Makiura, et al. 1974). Kimura et al demonstrated that pretreatment with Kanechlor-400 in diets of rats 4 months prior to and 2 months during treatment with 3'methyl-4-dimethylaminoazobenzene protected the rats against the formation of hepatocarcinomas induced by this carcinogen. PCBs were shown to add to the effects of BHC by Ito et al. (1973). These findings suggest that in terms of effects on tumorigenesis, timing is important in the application of PCB to the diets of laboratory animals. Treatment with the PCBs prior to the administration of a carcinogen can result in reduction of the tumor response, whereas treatment concurrent with a oncogen can result in enhancement of the tumorigenic response. The inhibitory effects of the PCBs were assumed to be due to the induction of hepatic microsomal enzymes, while the enhancement was ascribed to the additive hepatotoxic properties of the PCBs.

The two-stage system of mouse skin tumorigenesis allows one to evaluate critically the initiation and promotion phases of carcinogenesis individually. This system allows one to study the effects of modifiers on initiation and promotion seperately in a skin carcinogenesis assay. The results of Barry et al., (1979) demonstrate that PCBs possess the capacity to decrease tumor initiation in a mouse skin assay and that at the doses utilized, PCBs had no initiating or promoting properties. Barry, et al. (1979) tested PCBs for promoting activity in mouse skin with a high (200 nmol) initiating dose of DMBA. In a 30-week treatment period, the normal DMBA-initiated, TPA-promoted controls yielded approximately 8 papillomas per mouse. PCBs (at doses of 100 ug/mouse given twice weekly) did not promote the development of skin tumors. When tested without DMBA initiation, PCBs did not demonstrate any carcinogenic activity. PCBs did not produce any observable skin lesions. The anticarcinogenic effects of Aroc-

lor 1254 appear to correlate well with their ability to induce monooxygenase enzymes of the skin. These experiments suggest that pretreatment gives rise to an increased rate of inactivation of the DMBA molecule relative to the rate of activation in mouse skin. Although these data point to induction of oxidative biotransformation as a possible mechanism for the inhibitory effects exhibited, other mechanisms could be operating. Possibilities include induction in epidermal tissues of nonoxidative metabolic pathways such as epoxide hydratase, glutathione-S-transferase, UDP-glucuronosyltransferase and others. Other possibilities include effects on DNA-repair systems and on the distribution of the carcinogen to the critical target site(s).

8.12. Immunosuppressive Effects on Tumor Cells. PCBs have also been reported to act as immunosuppressive agents (Vos and Beems 1971, Vos and De Reij 1972, Keller and Thigpen 1973). Since a suppressed immunologic state in the host can enhance the generation and growth of tumors (Burnet 1970, Gatti and Good 1971, Penn and Starzl 1972, Heberman 1974), Kerkvleit and Kimeldorf (1977) conducted a study to determine the effects of host exposure to PCBs on tumor growth per se, utilising a transplantable tumor in rats.

Aroclor 1254, dissolved in corn oil, was incorporated into specially prepared powdered diets at levels of 1, 100, 400 or 800 ppm and fed to groups of male and female Sprague Dawley rats, eight of each sex per group. Subsequently three additional groups of 20 male rats were placed on diets containing 0, 5 or 25 ppm PCB. Following 30 days of exposure to the contaminated diets, the animals were inoculated with the Walker 256 carcinosarcoma. The tumors were allowed to grow for nine days, during which time the animals remained on their respective diets.

The mean tumor weights of all PCB-fed groups were significantly smaller than the mean tumor weight of their sex-matched control after the nine-day tumor growth period. In both male and female rats, the magnitude of tumor weight inhibition directly correlated with the concentration of PCB in the diet. Although female rats consistently showed smaller tumors than their male counterparts, the degree of tumor weight inhibition observed at each dose level was comparable in both sexes.

The results of this study demonstrate that PCB (Aroclor 1254) exposure can inhibit the growth of at least one experimental tumor, the Walker 256 carcinosarcoma in rats. The tumor inhibitory response of PCB was dose-related.

8.13. Are Chlorinated Hydrocarbons Different? Recent reviews of specific classes of chemical carcinogens indicate that correlation with mutagenesis depends, in part, on the chemical class (McCann et al. 1975, Andrews et al. 1978a & b, Ueno et al. 1978). It has been stated that certain compounds, especially persistent chlorinated compounds including PCBs, increase the incidence of only liver tumors in mice and that these tumors may not be malignant (Butler and Jones 1978). Halogenated chemicals are the most numerous class of chemicals to induce rat or mouse liver tumors alone and most are known not to be mutagenic. Nonmutagenic chemicals causing rodent liver tumors must act by epigenetic mechanisms.

The following facts emerge from the NCI bioassay program for polycyclic halogenated compounds:

1. 29 compounds studied by NCI representing 7 classes but excluding the cyclic

polychlorinated series, produced tumors in mouse liver as well as tumors at other sites;

2. 24 of these 29 compounds were positive in the Ames System;
3. Ten of 11 chlorinated polycyclic compounds tested by NCI produced only liver tumors in mice;
4. Four of the 10 chlorinated polycyclic compounds that produced liver tumors did so in only one sex;
5. None of the NCI tested chlorinated polycyclic compounds producing only tumors in mice produced tumors at sites other than the liver; and
6. One of the 8 tested compounds was positive in the Ames system.

When cyclic polychlorinated chemicals are excluded, 83% of the compounds producing tumors in mouse liver and at other sites are positive in the Ames system. Cyclic polychlorinated compounds producing either mouse and rat liver tumors in the NCI bioassay are positive in the Ames System only 12% of the time.

A review of the carcinogenic, mutagenic, and hepatotoxic literature reveals that PCBs meet many of the criteria for producing benign neoplastic lesion and therefore may be oncogenic. Cyclic polychlorinated compounds, however, produce tumors and Ames system responses different from most other carcinogenic compounds. PCBs are not positive in the Ames' system and do not produce tumors at sites other than the rodent liver. PCBs are unlikely to produce neoplastic effects through genotoxic mechanisms. Several carcinogenesis bioassays have been conducted and, with the exception of Kimbrough et al. (1975), all authors concluded that PCBs may be oncogenic but not carcinogenic.

8.14. Carcinogenicity Summary. The experiments conducted with PCBs reveal that:

1. PCBs cannot be initiators because PCBs are not mutagenic;
2. PCBs are not promoters of genotoxic initiators of liver tumors and, in fact, inhibit 3'-Me-DAB, 2-AAF and DENA;
3. PCBs are not promoters of DMBA in mouse skin and, in fact, inhibit the carcinogenic process;
4. PCBs act additively with the hepatotoxin BHC in increasing liver tumors;
5. PCBs do not enhance the carcinogenic process through impairment of the immune system as demonstrated by inhibition of Walker 256 carcinosarcoma cells;
6. Liver lesions produced by PCBs appear to have the capacity to regress;
7. PCBs only produce neoplastic lesions of the liver. No tumors have been produced at any other site;
8. Extraordinary levels of PCBs must be accumulated before neoplastic changes in the liver are observed;
9. Only one of many studies, that of Kimbrough et al. (1975), has reported PCB induced carcinoma. None of the tumors invaded or metastasized and the PCB used, Aroclor® 1260, was of unknown purity; and
10. When only liver tumors are produced by polycyclic chlorinated compounds, the relevance and correlation to human cancer risk is small.

Table 8 summarizes the carcinogenicity testing of PCBs.

8.15. Conclusion. The question is, does an experiment utilizing prolonged exposures at high levels to PCBs, which reportedly increases the incidence of liver

cancer in a single sex of a single strain, document a hazard of increased human cancer at environmental exposures. The conclusion of this review is that PCBs have not been demonstrated to be carcinogenic in an animal model of significant relevance to man, and do not present a cancer hazard at environmental levels of exposure.

9. EFFECTS OF PCBS IN HUMANS

Possibly the best evidence of what effects PCBs have in humans comes from the "Yusho" incident that occurred in Japan. In 1968, approximately 1,291 people in southwest Japan were affected by an exposure of 1-2 grams of PCBs that was ingested in a rice oil contaminated with a Japanese brand of PCBs known as Kanechlor 400 (Kuratsune 1976, Higuchi 1976, IARC 1978). For a breakdown of the symptoms observed, see Table 7.

Approximately half of the patients complained of a variety of neurologic distresses such as headaches, numbness, hypothesis and neuralgia. Most of the headaches were transient, but some recurred over a period of months to years. Higuchi (1976) has concluded that most of these probably arose from emotional stress or migraine conditions and were not related to PCB exposure. Measurements of nerve conduction velocities in Yusho patients revealed, however, that up to 50% of the 20 patients studied had lower than normal sensory nerve conduction velocities but normal motor nerve conduction velocities (Higuchi 1976).

Higuchi (1976) originally considered the possibility of adrenocortical hypofunction, but findings in the patients did not support this hypothesis and postmortem examinations did not reveal any unusual adrenocortical morphology.

Female patients, 60%, did suffer abnormal menstrual cycles but the changes were mixed; some suffered prolonged intervals of menstruation while others had shortened or irregular intervals. Determinations of urinary estrogens revealed a decrease; a possible explanation of this finding is an increased degradation of estrogens because of increased liver metabolism (Higuchi 1976).

Hematologic examinations of Yusho patients revealed a slight leukocytosis and monocytosis and serum levels of IgA and IgM were generally decreased. Surprisingly, serum indicators of liver damage such as GOT, GPT and bilirubin were normal. Thus, although PCBs can cause liver injury in experimental animals, as many halogenated hydrocarbons do, the clinical evidence at Yusho indicates that this is not a prominent toxicity in humans (Higuchi 1976, Kuratsune 1976). Nonetheless, microscopically observed changes have been seen in liver biopsy samples, but the major finding, an increase in the smooth endoplasmic reticulum, is interpreted as representive of the induction of the hepatic enzymes for oxidative metabolism, a finding predicted from animal studies and not an indication of toxicity. Analysis of other serum parameters also suggested a disruption of normal lipid metabolism. Serum triglycerides were elevated while cholesterol was not.

Of 43 affected children examined, 23 boys showed decreases in height and weight gain compared to unaffected children, while 19 girls did not differ from the control group (Higuchi 1976). Also, some of the infants born to women affected by Yusho were small-for-date. These two findings are felt to suggest that high doses of PCBs may retard growth in children. The other symptoms in newborns included dark-brown pigmentation, parchment like skin, eruption of teeth and larger than usual fontanelles indicating transplacental passage of PCBs to the fetus

MONS 016092

during gestation.

Probably the most common symptoms and the ones largely responsible for the identification of Yusho's disease were eye and skin problems. These consisted of acneform eruptions, follicular accentuation, swelling of the eyelids with discharge from the eyes and increased pigmentation of the skin. Unfortunately, even though the chloracne was not a permanent condition, it was a discomforting disfigurement that lasted for a period of months to years. (Kuratsune et al. 1972.)

While the above effects described in Yusho patients provide a picture of the possible consequences suffered by overexposure to PCBs, the exact cause of these disturbances is of some debate. The type of PCB mixture contaminating the rice oil, Kanechlor 400, has been discovered to contain polychlorinated dibenzofurans (PCDFs) at concentrations as high as 18 ppm (Kuratsune et al. 1972, Higuchi 1976, EPA 1980). Measurements of the rice oil used by Yusho patients revealed that the PCBs ranged from 1 - 3,000 ppm while the PCDFs were 5 ppm (Kuratsune 1976, Nagayama et al. 1976). The 2, 3, 7, 8, tetrachlorodibenzofuran isomer is an extremely toxic chemical causing liver damage, chloracne, birth defects and cancer in experimental animals (Huff et al. 1980). Therefore, it is not known which of the side effects seen in the Yusho disease can be attributed solely to PCBs (Vos et al. 1970) and, if cancer is found in the future to have been significantly increased in this group the source of induction will remain a question. (NOTE: The dibenzofurans found in the PCBs in Yusho incident are 1,000 times higher than those usually measured in PCBs).

Besides the Yusho incident, there are other recent reports in the literature

concerned with the effects of PCBs in humans. Ouw et al. (1976) studied 34 workers from a factory in which PCBs were added to capacitors. Air concentrations inside the plant previous to the study contained PCBs exceeding the allowable limit of 1.0 mg/m³ by 30-122% in some areas of the plant requiring the installation of a better exhaust system. Although the industrial hygiene of the plant had been less than desirable, with at least one case of chloracne and others reporting rashes or a burning of the eyes, the clinical tests did not reveal any significant health problem. PCB blood levels of the group averaged over 400 ppb compared to no detectable amounts in the control population, yet the bilirubin, alkaline phosphatase, serum protein, albumin, SGPT and immunoglobulins of the workers were all within normal limits.

Fischbein et al. (1979) have also examined workers employed at a capacitor plant. In their study, 326 employees were examined and of these the breakdown for the duration of exposure was: 10% had 5 or less years, 20.9% had 5-10 years, 17.5% had 10-15 years, 11.4% had 15-20 years, 29.1% had 20-25 years and 11% had greater than 25 years of exposure to PCBs. Of these employees 10.7% had reported rashes and 24.8% had reported a burning sensation of the skin. Upon physical examination, approximately 40% of the group had some redness, swelling, dryness or thickening of the skin and 2% had abnormal secretions from the eye. Overall, the clinical chemistry of the employees was unremarkable and this "paucity of abnormal results" was noted by the authors. Routine neurologic examinations also did not reveal any remarkable prevalence of abnormalities. There was, however, a decrease in the forced vital capacity of the lungs in 14% of the workers compared to 5.6% in the normal population, an unusual finding but one of unknown significance at this time (Warshaw et al. 1979).

Other studies concerned with employee exposure have been adequately discussed elsewhere (NIOSH 1977, EPA 1980). These discussions reveal that the toxicities most often found after occupational exposure are related to the dermal changes discussed and indicate little or no liver injury or other systemic problems. While these studies indicate that the liver injury seen in animals has not been correspondingly reflected in humans at the levels of PCB exposure generally encountered occupationally, a study by Alvares and co-workers (1977) suggests these concentrations may indeed increase liver metabolism. The antipyrene half-life measured in five workers exposed to PCBs for at least four years was two thirds that of the half-life of the control group leading to a 30% increase in antipyrene clearance.

Reports of high cancer rates among Mobil Oil employees at its Paulsboro, NJ refinery exposed to PCBs (Aroclor 1254) have been interpreted as indicative of to a possible link between PCB exposure and skin (melanoma) or pancreatic cancer (Bahn et al. 1976). The Mobil study indicated that 8 cancers developed between 1937 and 1975 among 92 research and development and refinery workers exposed for 5 or 6 years in the late 1940's and early 1950's to varying levels of Aroclor 1254. Of the 8 cancers, 3 were malignant melanomas and 2 were cancers of the pancreas. NIOSH said, "This is significantly more skin cancer (melanoma) and pancreatic cancer than would be expected in a population of this size, based on the Third National Cancer Survey." However, it is difficult to derive any conclusions from this study because of the small numbers of individuals exposed and the variety of other agents to which they were exposed.

It should be noted that Monsanto Co., in contrast to Mobil Oil, could find no casual relationship between cancer and PCB exposure at its plant in Sauget, Illi-

nois. The Monsanto study was based on a review of the records of more than 300 current and former employees at the Illinois plant that had been engaged in PCB production since 1936 (Anon 1976).

Findings of an increased risk of mortality due to malignant melanoma, cancer of the pancreas, and lung cancer among workers exposed to PCBs was not corroborated in the study of Brown and Jones, (1981). There were no observed deaths due to malignant melanoma and only 1 observed death from pancreatic cancer, while 1.89 were expected. There were seven observed deaths from respiratory system cancer, whereas 7.69 were expected. There was no total relationship between increasing durations of employment in jobs involving PCB exposure and the risk of mortality due to cancer or cirrhosis of the liver in the Brown and Jones study.

9.1. Summary of Human Health Effects. The literature on human toxicological effects on reproduction, birth defects, mutagenicity and general toxicity, has been reviewed, as well as the literature relevant to human occupational exposure and accidental poisonings. The following conclusions have been made: PCBs represent a low, acute, exposure hazard; mutagenic, teratologic, and reproductive risks are minimal; metabolism in man is likely less aggressive than in rodents; and the low levels of PCBs generally experienced in the environment pose little risk and no obvious hazard. However, there have been reports of PCBs containing highly toxic contaminants, e.g., polychlorinated dibenzofurans, and the presence of these contaminants could modify PCB toxicity and the health risks associated with exposure.

10. SUMMARY AND CONCLUSION

Poisons are agents that can produce adverse effects on a biological system. Adverse effects may vary from an alteration of normal function to the destruction of life. All chemicals are capable of altering some function in some organism at a large enough dose; therefore, all chemicals could be defined as poisons. Because the definition of a poison can be broad and does not describe those circumstances and conditions under which an adverse effect can be expected to be produced, a more useful definition of the toxicity of a chemical focuses on those conditions predicted to develop at likely exposures to the chemical. Evaluation of experimental evidence and establishment of relevance to man is necessary before defining risk or hazard to man and the magnitude of that risk or hazard. Safety is relative and is defined as the probability that a substance will not produce an unacceptable alteration of normal function under a given set of specified conditions. The success of predictions of safety depends on the types of experiments performed, the adequacy with which they have been performed, and, most importantly, the appropriateness of extrapolating from experimental results to man. To maximize the utility of the data, the toxicity of the material should be determined under controlled circumstances relevant to defining the minimum conditions necessary to produce adverse effects.

PCBs can be toxic. PCBs can be used safely under controlled conditions. PCBs under known conditions of exposure to man, have not, with the exception of overt poisonings, produced significant adverse health effects. The literature of PCB toxicity in both animals and humans has been reviewed to predict those conditions under which PCBs may be considered poisonous or unsafe. Included in the literature review were studies on reproduction, birth defects, mutagenicity,

carcinogenicity and general systemic toxicity. In addition, the literature was reviewed for data relevant to human occupational exposure and accidental poisonings. Analysis of published animal data leads to the conclusion that, in animals, pure PCBs represent a low acute exposure hazard, that mutagenic, teratogenic and reproductive risks are minimal, and that the carcinogenic potential of this compound has not been convincingly demonstrated in an animal model relevant to man.

The metabolism of PCBs in man is much less aggressive than in rodents, therefore bioactivation models proposed as explaining chronic toxicities in rodents are inappropriate to predict similar hazards in man. Analysis of human PCB exposure and effect data reveals a spectrum of toxicities fairly consistent with those induced in animal tests. Acute human exposures have not produced the significant liver damage seen in chronic exposures of rodents, as evidenced by the Yusho incident. Human occupational epidemiology studies with substantial numbers of exposed workers indicate minimal systemic toxicity and no increase in cancer incidence. PCBs represent no unusual hazard when compared to many "safe" chemicals with the exception of chloracne and adverse dermal responses. Human exposure to the PCBs that are generally present in the environment is at much lower levels and/or for shorter periods of time than those exposures documented for Yusho or in capacitor plants. Therefore, it is concluded that low levels of PCBs pose little risk and no obvious hazard.

The chemical analyses of PCBs have shown that they often contain polychlorinated dibenzofurans (PCDFs) at low levels. The concentrations of these toxic contaminants are generally in the parts-per-million range in pure PCB mixtures, but percent of contamination can be substantially increased as a result

of industrial use. Since levels of PCBs generally found in the environment are in the parts-per-million range or lower, the concomitant concentrations of PCDFs would be expected to be unmeasurable. For this reason and because the toxicity data of all PCBs exposures have probably included this contaminant, the concern for low level exposures to PCDFs is still expected to be minimal. Although the presence of these contaminants could modify the toxicity of PCBs and the health risks associated with exposure, their expected low levels should represent no unusual hazard.

List of Tables

Table 1

ISOMERS OF PCBs

# Chlorines	Chlorobiphenyl	# Isomers	Molecular Weight	% Chlorine by Weight
1	mono	3	188.7	18.79
2	di	12	223.1	31.77
3	tri	24	257.6	41.30
4	tetra	42	292.0	48.56
5	penta	46	326.4	54.30
6	hexa	42	360.9	58.93
7	hepta	24	395.3	62.77
8	octa	12	429.7	65.90
9	nona	3	463.1	68.73
10	deca	1	498.5	71.76

Adapted from Hartzinger et al., 1976, NIOSH 1977 and IARC 1978.

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Table 2

PHYSICAL PROPERTIES OF COMMERCIAL PCBs

Property	Aroclors[a]					
	1221	1016	1242	1254	1260	1260
Appearance	clear, mobile oil	clear, mobile oil	clear, mobile oil	light yellow, thick oil	sticky resins	sticky resins
Specific Gravity @ 25°C	1.18	1.36	1.38	1.58	1.57	1.61
Distillation Range (°C)	275-320	323-356	325-366	365-390	385-420	435-450
Vaporization Rate at 100°C (mg/cm²/hr) -or- % loss @ 100°C/6 hr	1.74	---	0.34	0.05	---	---
	---	---	---	0-0.2%	0-0.1%	0-0.1%
Water Solubility at 25°C (mg/l)	---	---	---	0.4	---	---

[a] Trademark of Monsanto
Adapted from Hartzinger et al 1974, NIOSH 1977 and IARC 1978

MONS 201910

Table 3

END USES OF AROCLORS* BY TYPE

End use	1016	1221	1232	1242	1248	1254	1260	1262	1268
Current									
- Capacitors	x	x		x	x				
- Transformers				x		x	x		
Former									
- Heat Transfer				x					
- Hydraulics/Lubricants									
- Hydraulic Fluids			x	x	x	x	x		
- Vacuum Pumps					x	x			
- [illegible]Gas-transmission Turbines		x		x					
- Plasticizers									
- Rubbers		x	x	x	x	x			x
- Synthetic Resins					x	x	x	x	x
- Carbonless Paper				x					
- Miscellaneous									
- Adhesives		x	x	x	x	x			
- Wax Extenders				x		x			x
- Reducing Agents						x	x		
- Inks						x			
- Cutting Oils						x			
- Pesticide Extenders						x			
- Sealants and Caulking Compounds						x			

* Trademark of Monsanto
Adapted from Hutzinger et al 1974 and IARC 1978

MONS 016103

Table 4

PCBs IN FOOD

Products	Percent of samples with PCBs 1973	1974	1975	Maximal Concentration (ppm)
Fish	60.6	44.0	17.8	123.0
Milk	2.2	2.6	0.7	2.3
Eggs	1.1	4.2	0.0	11.0
Cheese	0.9	2.6	0.0	2.8
Feed Components	12.7	0.0	0.3	9.0
Animal Feed	7.2	0.0	0.0	199.5
Processed Fruit	4.5	0.0	0.0	19.2
Baby Food	1.1	0.0	0.0	trace
Meats, Poultry	1.9	1.2	0.3	> 5 ppm

Adapted from Jelinek and Corneliussen 1976.

Table 5

TOXICITY RATING CHART

Rating	Probable Oral Lethal Dose for Humans*	
	Animal LD_{50}	Expected Human Dose
1. Nontoxic	15,000 mg/kg	≥ 1 Quart
2. Weakly Toxic	5,000-15,000 mg/kg	1 Pint-1 Quart
3. Moderately Toxic	500-5,000 mg/kg	1 Ounce-1 Pint
4. Toxic	50-500 mg/kg	1 Teaspoon-1 Ounce
5. Extremely Toxic	5-50 mg/kg	7 Drops-1 Teaspoon
6. Supertoxic	≥ 5 mg/kg	Less Than 7 Drops

* Average Adult of 70 kg
Adapted from Doull et al. 1980.

Table 6

ACTUAL TOXICITY AND THE TOXICITY RATING CHART

OF SOME SELECTED CHEMICALS

Agents	Animal LD_{50}	Expected Human Dose*
PCBs	14,000 mg/kg	1 Quart
Alcohol	10,000 mg/kg	1 Pint-1 Quart
Table salt	4,000 mg/kg	1 Pint
Iron	1,500 mg/kg	1 Ounce-1 Pint
DDT	100	1 Teaspoon-1 Ounce
Strychnine	2	4 Drops
Nicotine	1	1 Drop
TCDD	0.001	Less Than 1 Drop
Botulinus toxin	0.00001	Less Than 1 Drop

* Average Adult of 70 kg
Adapted from Doull et al. 1980.

Table 7

GENERAL SYMPTOMS OF "YUSHO" PATIENTS

Subjective Symptoms	Objective Symptoms	Clinical Results
1. General fatigue	1. Bronchitis-like	1. High PCB Concentration in fat and blood
2. Headache	2. Sensory neuropathy	2. Increase of serum neutral lipids.
3. Abdominal pains	3. Buratis	3. Anemia
4. Numbness or pain of the limbs	4. Inhibition of growth in children	4. Reduced conduction velocity of sensory nerves.
5. Swelling and pain in the joints	5. Small for date babies	5. Adrenocortical hypofunction
6. Cough and sputum	6. Acneform eruptions on skin.	
7. Changes in menstruation	7. Increased pigmentation of gingiva, skin and nails.	
	8. Discharge from meibomian glands.	
	9. Edema of face and eyes	
	10. Liver enlargement	

Adapted from Kuratsume 1976

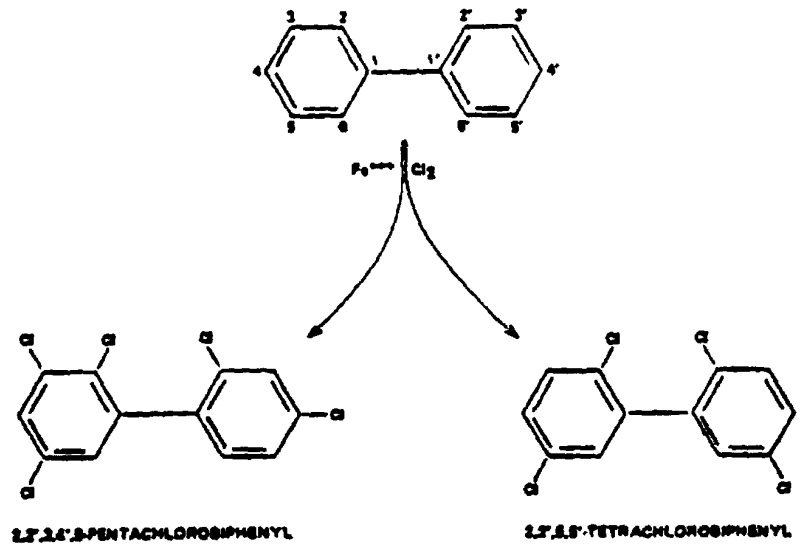
MDNS 016107

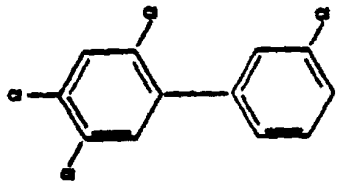
Table 8

TUMORIGENIC EFFECTS OF PCB's

AUTHORS	YEAR	SPECIES	COMPOUND, DOSAGE	EFFECTS
Ito et al.	1973	Mice	Kanechlor-500 in diet 500 ppm/12 m	Nodular hyperplasias and well differentiated hepatocellular carcinomas in the liver. Additive of carcinogenesis by α-BHC and β-BHC.
Kimbrough et al.	1974	Mice	Aroclor 1254	Hepatomas and adenofibrosis
Nagasaki et al.	1974	Mice	Kanechlor-500 500 ppm/ α-BHC 250 ppm/24 wk Kanechlor-500 100 ppm + α-BHC 50 ppm	32 wk Neoplastic changes in liver 32 wk Neoplastic changes in liver 32 wk Neoplastic changes in liver
Nagasaki et al.	1975	Mice	Penta- tetra- or trichloro-biphenyls, 100, 250, 500 ppm for 24 wk Penta- and tetra-chloro-biphenyl 100 ppm + 50 ppm α-BHC	Liver tumorigenesis in higher incidence at higher dose rates. Liver tumorigenesis of α-BHC was promoted also by pure chlorinated biphenyls.
Kimura & Babe	1973	Rats	Kanechlor-400 (1000-1500 mg--the total for 400 days)	Neoplastic nodules change in 6/10 females and 8/10 males.
Makiura et al.	1974	Rats	Kanechlor-500	Inhibited the induction of liver tumors by several liver carcinogens.
Kimbrough et al.	1975	Rats(F)	Aroclor 1260 100 ppm/21/m	Hepatocellular carcinomas 26/104 Hyperplastic nodules 146/104 Adenofibrosis
Wassermann et al.	1978	Rats(F)	Aroclor 1254 200 ppm/25 m	Hepatocellular adenomas.
Kimura et al.	1976	Rats	PCB's	Anti-promoters in experimental carcinogenesis
Allen & Norbach	1973	Rhesus Monkeys	Aroclor 1248 300 mg/kg PCB's 5000 mg/kg	Hyperplasia and dysplasia of gastric mucosa with invasion of adjacent tissues.
Allen & Abishamson	1973	Rats	Aroclor 1240, 1254, 1262	Hypertrophy - regressive changes
Kimbrough & Linder	1977	Mice	Aroclor 1254	Regression of hepatomas
Nagasaki et al.	1972	Mice(M)	Kanechlor 500	Male hepatomas (400 + 300 Negative)
Kerkvliet & Kimeldorf		Rats	Aroclor 1254	Inhibited Walker 256 carcinosarcoma
Ito et al.	1974	Rats	Kanechlor 500, 400, 300	Nodular hyperplasia, cholangiofibrosis
Burse et al.		Rats(M)	Aroclor 1242, 1016	Liver cell necrosis
Kimbrough et al	1972	Rats(M & F)	Aroclor 1260, 1254 20-1000 ppm	1254 more pronounced contaminated dibenzofuran

MONS 016108





2,3',4,5-TETRACHLOROBIPHENYL

NOT

2,4,5',5-TETRACHLOROBIPHENYL
NOR
3',3,4',5'-TETRACHLOROBIPHENYL

Figure 1 PCB STRUCTURES AND NOMENCLATURE

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MONS 016121

Glossary

GLOSSARY OF TERMS

Oncogen	see page 28
Carcinogen	see page 29
Teratogen	agent causing abnormal development of embryo or fetus
Mutagen	any change in the character of the gene that is perpetuated in subsequent cell divisions
kg	kilogram (2.2 pounds)
g	gram (1/1,000 of a kilogram)
mg	milligram (1/1,000 of a gram)
ug	microgram (1/1,000,000 of a gram)
ng	nanogram (1/1,000,000,000 of a gram)
l	liter (0.91 quarts)
m^3	cubic meter (of air)
nmol	nanomoles (1/1,000,000,000 of a mole of a substance)
$1mg/m^3$ of PCP	approximately equals 0.0615 ppm (assuming a molecular weight of 300)
NIOSH	National Institute of Occupational Safety and Health
NCI	National Cancer Institute
EPA	Environmental Protection Agency
IARC	International Agency for Research on Cancer

Biographical Information on the Authors

MONS 016124

BIOGRAPHICAL SKETCH OF AUTHORS

Robert C. James, PhD

Dr. James is the Corporate Toxicologist for Ecology and Environment, Inc. He is responsible for assessing the impacts of toxic and hazardous materials on the environment and on human health. Dr. James has had several years experience making such assessments as well as developing technical transfer documents and courses concerned with mutagenicity, carcinogenicity, hazardous materials, hazard evaluation/risk assessment, industrial toxicology, and the basic principles of toxicology for sponsors like Vanderbilt University, Georgia Institute of Technology and the United States Environmental Protection Agency. In addition, Dr. James has conducted basic research in toxicology and published over 20 articles in the areas of xenobiotic metabolism, regulation of metabolism during chemical injury, the disposition of chemicals, and chemically induced toxicities caused by metabolic activation.

Dr. Morris Cranmer, PhD

Dr. Cranmer is currently the President of Jefferson Professional Services, which provides consulting, testing and management services to clients worldwide. He is also a Professor in the Department of Interdisciplinary Toxicology at the University of Arkansas. His expertise in the field of toxicology lies mainly in these areas of: techniques for identifying carcinogens; risk estimation procedures; fate and effects of chemicals in the environment; management of applied research laboratories; and federal environmental and health effects legislation. In the course of his work he has published over 150 papers, books, manuals and presentations. In past years he functioned not only as Director of

the National Center for Toxicological Research, but he has also served with the Environmental Protection Agency, the Food and Drug Administration, the Center for Disease Control, and at the University of Miami and Michigan. He is affiliated with thirteen scientific or professional societies and is on the editorial board of four scientific journals. As a scientist, he has received additional recognition by having received the highest awards given by the Department of Health, Education and Welfare (the Distinguished Service Award, 1976); the Society of Toxicology (National Achievement Award, 1976); and the Food and Drug Administration (Award of Merit, 1973).

Raymond Harbison, PhD

Dr. Harbison is currently Chairman of the Interdisciplinary Toxicology Program at the University Arkansas. His expertise in the field of toxicology covers the areas of: terstology (i.e. birth defects as well as reproductive, embryo and fetal toxicology); bioactivation and detoxification mechanisms; hazard/safety analysis of toxic wastes; fate and effects of chemicals in the environment; and industrial/occupational health and safety. Dr. Harbison has previously taught at both Vanderbilt and Tulane Medical Schools and developed the National Hazardous Materials Training program for the Environmental Protection Agency which has been also used to train the U.S. Coast Guard, Navy and industrial clients in the safe handling of toxic materials. Dr. Harbison has published over 90 papers, books and presentations and currently serves on the editorial board of three scientific journals. He is affiliated with seven different scientific and professional societies. Dr. Harbison has received further recognition by his peers, in having received the Society of Toxicology's National Achievement Award in 1978.

MONS 016126