III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Biphenyl (C 12H 10), diagrammed in Figure III-1, can be chlorinated by replacing any or all of its hydrogen atoms with chlorine [1].

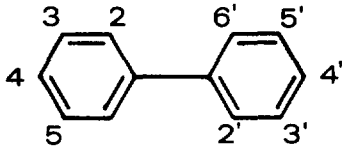
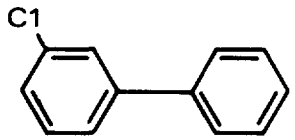
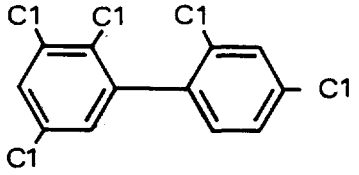


Figure III-1. BIPHENYL MOLECULE AND RING NUMBERING SYSTEM

Specific chlorobiphenyl molecules are designated by reference to the positions of the chlorine atoms according to the numbering scheme depicted in Figure III-1. The lowest possible numbers are assigned, and the phenyl moiety with the fewest chlorine atoms is assigned prime numbers [1,2]. Examples of the nomenclature used here are shown in Figure III-2.



3-chlorobiphenyl



2,2',3,4',5-pentachlorobiphenyl

Figure III-2. EXAMPLES OF NOMENCLATURE SYSTEM OF CHLOROBIPHENYL COMPOUNDS

There are three monochlorobiphenyl compounds, 2-, 3-, and 4-chlorobiphenyl. The 5- and 6-monochlorobiphenyls are identical to 3- and 2-monochlorobiphenyl, respectively. There are 18 dichlorobiphenyl

compounds. The number of possible chlorobiphenyl isomers and the corresponding weight-percents of chlorine are presented in Table III-1.

TABLE III-1

NUMBER OF ISOMERS AND PERCENT CHLORINE
FOR THE 10 CHLOROBIPHENYL (PCB) CLASSES

Chlorobiphenyl	Empirical Formula	No. of Isomers	Weight % Cl
mono	C 12H9C1	3	18.79
di	C 12H8C1 2	12	31.77
tri	C 12H7C1 3	24	41.30
tetra	C 12H6C1 4	42	48.56
penta	C 12H5C1 5	46	54.30
hexa	C 12H4C1 6	42	58.93
hepta	C 12H3C1 7	24	62.77
octa	C 12H2C1 8	12	65.98
nona	C 12HC1 9	3	68.73
deca	C 10C1 10	1	71.18

Adapted from reference 1

In the commercial synthesis of chlorobiphenyls, biphenyl is catalytically chlorinated with anhydrous chlorine; either iron filings or ferric chloride may be used as the catalyst [1]. The commercial preparations, commonly referred to as "PCBs," are isomeric mixtures. The weight-percent chlorine in commercial mixtures has generally varied between 21 and 68% and has been used to designate grades of commercial products. Commercial PCB products manufactured in the US, Great Britain, and Japan have been marketed under the trade name "Aroclor" [1-3]. Several grades of Aroclor have been designated by numbers such as 1221, 1242, 1254, and 1260, where the last two digits represent the percent by weight of chlorine in

the mixtures. Another grade of Aroclor, 1016, made primarily of tri- and tetrachlorobiphenyl compounds and containing 41% chlorine by weight, was introduced in 1971 to replace Aroclor 1242 [2,4,5]. Other PCB products manufactured in Japan were marketed as "Santotherm" [1], and "Kanechlors" 300, 400, 500, and 600, containing approximately 42%, 48%, 54%, and 60% chlorine, respectively [6]. In Germany, products marketed as "Clophens" A50 and A60 contained 54 and 60% chlorine, respectively [7]. In France, PCBs were marketed as "Phenoclors" and "Pyralenes;" Phenoclor DP6 contains 60% chlorine [8]. Other countries reported to have produced PCBs are Italy, Spain, Czechoslovakia, Poland, Argentina, Brazil, the USSR, and India [1,9,10].

The chlorobiphenyl constituents of several commercial PCB products have been studied [6-8,11-16]. Some data, both qualitative and quantitative, are presented in Table XII-2. About half the 209 possible chlorobiphenyls do not occur in any of the commercial preparations. Among those compounds which do not occur, or which occur in trace amounts only, are 3-chlorobiphenyl, all of the tri- to pentachloro compounds that are chlorinated in only one ring, the penta-, hexa-, and heptachloro compounds that are completely chlorinated in one ring, and the penta- and hexachloro compounds that are chlorinated in four positions in one ring.

Commercial PCBs are insoluble in water, but are soluble in oil and many organic solvents. Some other physical and chemical properties of certain Aroclor products are presented in Table XII-1 [1].

In addition to chlorinated biphenyls, the commercial mixtures with 20-40% chlorine contained biphenyl from about 11% to traces, respectively, by weight. Many commercial PCB products also contain chlorinated

dibenzofurans [17-23] and naphthalenes [18,19]. Concentrations of chlorinated dibenzofurans in various commercial PCB products are presented in Table III-2.

TABLE III-2

CHLORODIBENZOFURAN TYPES AND CONCENTRATIONS (μg/g) IN COMMERICAL PCB PREPARATIONS

Mixture*	Chlorodibenzofurans di	tri	tetra	penta	hexa	hepta	Total	Ref.
(1) 1016	0.5						0.5	**
(1) 1016			<0.001	<0.001	<0.001			21
(1) 1248			0.5	1.2	0.3		2.0	21
(1) 1254			0.1	0.2	1.4		1.7	21
(1) 1254			0.2	0.4	0.9		1.5	21
(1) 1260			0.1	0.4	0.5		1.0	21
(1) 1260			0.2	0.3	0.3		0.8	21
(2) A-60			1.4	5.0	2.2		8.4	21
(3) DP-6			0.7	10.0	2.9		13.6	21
(4) K300			(a)	(a)			1-1.5	20
(4) K400	(c)***	(e)	(e)	(c)			17-18	20
(4) K500				(a)	(c)	(a)	2.5-4	20
(4) K600			(a)	(a)	(b)	(b)	3-5	20

*(1) Aroclor, (2) Clophen, (3) Phenoclor, (4) Kanechlor
**(I Pomerantz, written communication, January 1977)
*** (a), (b), (c), (d), (e) represent relative amounts in increasing order

Some commercial preparations that were marketed under the trade name Aroclor contained chlorinated terphenyls in addition to chlorinated biphenyls. Aroclors 2565 and 4465 contained 75% and 60% chlorinated biphenyl compounds and 25% and 40% chlorinated terphenyl compounds, respectively. Both mixtures contained 65% chlorine [1].

A broad class of nonflammable synthetic chlorinated hydrocarbon insulating liquids used in electrical capacitors, transformers, nuclear

reactors, and accessory equipment is designated by the generic term "askarel" [4,5,24,25]. PCBs have been major components of most askarels used in the US since 1932. Two general classes of PCB-containing askarels are "capacitor"-grade and "transformer"-grade [4,5]. Aroclor 1242 was the major capacitor impregnant in the US before 1971; subsequently, Aroclor 1016 has been used mainly for this purpose [4]. Transformer-grade askarels manufactured in the US include those marketed under trade names including "Asbestol," "Chlorextol," "Inerteen," "No-Flamol," "Pyranol," and "Saf-T-Kuhl" [4]. Transformer-grade askarels are usually mixtures of trichlorobenzene and more highly chlorinated (42-60%) biphenyls [5,24]. Some typical compositions are: 100% Aroclor 1242; 70% Aroclor 1254 and 30% trichlorobenzene; 60% hexachlorobiphenyl and 40% trichlorobenzene; 45% hexachlorobiphenyl and 55% trichlorobenzene; and 70% pentachlorobiphenyl and 30% trichlorobenzene. Another type of transformer-grade askarel contains 45% polychlorinated biphenyl (54% chlorine) and 55% of a mixture of tri- and tetrachlorobenzenes. Transformer-grade askarels also contain stabilizers such as glycidyl phenyl ether and 3,4-epoxycyclohexylmethyl-3, 4-epoxycyclohexane carboxylate [24].

Exposure to commercial preparations of PCBs in the work environment may involve many different chlorinated biphenyl compounds, and also substantial amounts of biphenyl, tri- and tetrachlorobenzenes, and small amounts of many different chlorinated dibenzofurans, chlorinated naphthalenes and in special uses, chlorinated terphenyls.

Commercial production of PCBs in the US began in 1929 and reached 85 million pounds in 1970 when the major producer began selling PCBs only for use in "closed" systems. Since 1972, only those Aroclors designated 1016,

1221, 1242, and 1254 have been produced in the US, and total annual production has been around 40 million pounds [26].

Imported PCBs amounted to about a half-million pounds in 1972 and 1973 [26,27]. A decachlorobiphenyl produced in Italy and imported by one company for use in investment casting waxes accounted for 80-90% of the total PCB imports [26]. The other 10-20% was imported from France for use in semiclosed heat transfer applications [27]. Occupational exposure to decachlorobiphenyl could occur in the manufacture of the investment casting waxes as well as in the preparation of the investment casting molds in the 25 US foundries which use the decachlorobiphenyl wax [26].

Most of the PCBs produced in the US since 1971 have been used in electrical capacitors (70%) and transformers (30%). About 95% of the 100 million capacitors produced annually in the US contain PCBs [27]. There is potential occupational exposure to PCBs in the plants of the 17 reported companies that manufacture capacitors in the US. Capacitors are generally classified into two categories for disposal purposes. "Small" capacitors contain less than 2 pounds of PCBs; those incorporated into electrical equipment such as television sets, home air conditioners, and light fixtures contain 2-340 ml of PCBs and have service lives of at least 10 years [26,28]. "Large" capacitors may contain about 25 liters of PCBs [26] and have a service life of 15-20 years [26,27]. Potential occupational exposures exist in the servicing of appliances and in the disposal of used capacitors or equipment.

Transformers that contain PCBs are used mainly in or near inhabited buildings where fire hazards from cheaper oil-filled transformers are greatest. The estimated 135,000 PCB-containing transformers represent

about 5% of all transformers in the US [27]. Occupational exposure to various askarels used for transformers may occur in their manufacture, servicing, and transportation, or as a result of leaks [29].

Other potential occupational exposures to PCBs exist through losses in storage [30], shipment [29], manufacture and use of heat exchange units [27,31], and in use of previously manufactured items which contain PCBs, such as hydraulic systems, vacuum pumps, and gas transmission turbines [30,31]. The past use of PCBs in carbonless copying papers may result in exposure of workers currently engaged in paper reclamation [30]. Workers in plants that previously used PCBs may have current exposure in their working environments because PCBs have been shown to remain in the workplace air and on surfaces for years after PCB use has been discontinued [32,33]. Several occupations that may have involved exposure to PCBs were tabulated in a 1966 publication [34]. NIOSH estimates that 12,000 workers have potential occupational exposure as a result of current uses of PCBs in their working environments [35].

In addition to their occupational exposures, PCB workers may be exposed to PCBs carried into their homes from the workplace [36], from general contamination of the ambient air [26,37,38] and water [26,37], and to PCBs and their metabolites in their diets [39-46].

Metabolism and Mechanism of Action

A study of the metabolism of 4-chlorobiphenyl was reported in 1959 by Block and Cornish [47]. In this experiment, 1 gram of 4-chlorobiphenyl was fed to rabbits in a single dose and 4'-chloro-4-biphenylol and its

glucuroniside were recovered from the urine in amounts that accounted for 24 and 50%, respectively, of the administered dose.

Subsequently, a substantial number of metabolic studies of individual chlorobiphenyl compounds [41-43,48-84], mixtures [85,86], and metabolites [48,49,87] were reported, and a comprehensive review was published in 1976 [88]. These reports collectively demonstrate through many study methods that some aspects of metabolism are of special significance to the toxicity of PCBs and that some isomers are more toxic, or have different effects, than others [89-94].

Chlorinated biphenyl compounds are readily absorbed from the digestive tract, regardless of the degree or pattern of chlorine substitution [50,51,95,96]. It seems likely that similar absorptive characteristics apply to the respiratory system since chlorobiphenyl mixtures in air are rapidly absorbed [97], as is decachlorobiphenyl [98,99]. Varying amounts of chlorobiphenyls, depending on degree and pattern of chlorination, are excreted in the feces [42,50,53-56,100], milk [41-44,51,101], and hair [102] of animals, but no more than trace amounts are excreted in the urine [42,51-55,57-62,103-105].

Metabolites of chlorobiphenyl compounds have been found in the urine of mammals including mice [52], rats [48,53-56,58-60,63,64,66,71,85], rabbits [65,67-69], monkeys [51,61,74,75], swine [72], goats [73], and cows [41,73], as well as in feces [48,50-54,56,58-65,76-80], and milk [41]. The metabolites excreted in urine [53,54,58,59,61,63,64,66,67], bile [50,51,53,81], feces [52-54,58,63,76], and milk [41] are, to varying degrees, conjugated with glucuronic or sulfuric acids. Differences in metabolism of PCBs among the aforementioned species are more quantitative

than qualitative [51,73]; however, metabolism in birds and fish may differ qualitatively from that in mammals [55,77]. Among mammals there are also quantitative differences in PCB metabolism and in effects related to age and sex [50,93,106]. Metabolites identified in mammals include mono- to polyhydroxylated derivatives [41,48,49,51-56,58-81,85], and methoxy [59,60], hydroxymethoxy [64,65,68,70,76,87], dihydrodihydroxy [51,53,54,69,74,75,81], hydroxydihydrodihydroxy [51,74], and dechlorinated derivatives [48,65,70,77].

The lower-chlorinated biphenyl compounds are more readily metabolized than are the more highly chlorinated ones [41,50,51,55,61-64,66,82-84,96,107-109], with no metabolism having been demonstrated for decachlorobiphenyl [66]. As a consequence, some of the more highly chlorinated compounds persist in the tissues for years after intake has been discontinued [51,83,103,110].

The presence of at least two adjacent, unsubstituted hydrogen atoms, particularly in positions 3, 4, and 5, or 3', 4', and 5', is required for rapid metabolism of chlorobiphenyls [79,83,84]. All mono- di-, and trichlorobiphenyls, and all tetrachlorobiphenyls except 3,3',5,5'-tetrachlorobiphenyl meet this requirement. The latter compound, 3,3',5,5'-tetrachlorobiphenyl, was found to be particularly toxic to monkeys, and it was suggested that the chlorinated dibenzofuran derivative may have been involved [94]. While dibenzofuran derivatives have not been demonstrated to exist as mammalian metabolites, they may have been identified as PCB metabolites in chickens [111] and Curley et al [22] reported the excretion of dibenzofurans in urine of rats. In the latter case, however, dibenzofurans were also identified in the administered PCB [22].

Dibenzofurans are of concern because they may be many times more toxic than PCBs [19,112].

Metabolites found in urine [48,51,53,56,60,61,64,66-74,85], bile [81], feces [51-53,56,58,60,61,64,66,76,79,80,113], and milk [41] include hydroxy derivatives. Such compounds have been demonstrated to be more toxic than their respective parent chlorobiphenyl [41,114] and their presence in milk [41] is of special concern.

Hydroxylation may be direct through hydroxylating enzyme systems, or through formation of arene oxide intermediates [48,51,53,68,75,81,87,115]. This latter process is of particular concern because of potential carcinogenesis and mutagenesis as a result of covalent binding of arene oxides to nuclear components of the cell [51,68,74,82,86,116]. Such binding has been demonstrated both in vivo [51,86] and in vitro [51,82,86,116,117]. Additional evidence of hydroxylation through arene oxide intermediates included findings of metabolites in which chlorine, dueterium, or tritium were at different molecular locations than in the administered compound [68,115,116,118], and transdihydrodiols as mammalian metabolites [51,69,74,75,81].

Although adjacent unsubstituted hydrogen atoms are necessary for rapid metabolism of chlorinated biphenyls, it has been demonstrated that 2,2',4,4',5,5'-hexachlorobiphenyl, which does not have this characteristic, can be hydroxylated [70,79], and oxidatively dechlorinated [65,70]. It has been proposed that the metabolism of this compound may also involve arene oxide formation [65], and chronic exposure to potential carcinogenic activity of resulting arene oxides [86] may result from the metabolism of this and similar compounds [75].

Historical Reports

Smyth [119], in a paper read October 28, 1930, presented the results of his studies with biphenyl, 2- and 4-chlorobiphenyl, and two unidentified polychlorobiphenyl mixtures. He reported the oral minimum lethal doses for rabbits and guinea pigs as 4+, 2.5, 3.5, 4+, and 4+ g/kg, respectively.

Health problems associated with the manufacture of PCBs were the subject of a report by Jones and Alden [120] in 1936. The case history was presented of a man whose employment, from April 1930 to the end of 1933, involved the distillation of chlorobiphenyl. In May 1933 he developed chloracne, a specific type of acne known to be caused by some chlorinated hydrocarbon compounds [121,122]. Jones and Alden [120] stated that the manufacturing process was not enclosed, that a different source of benzene (a starting ingredient) had been used from the summer of 1932 through October 1933, and that from March to October 1933, the dielectric qualities of the PCBs produced had been substandard. Of 24 men working in the manufacturing process during the period beginning in the summer of 1932, 23 developed chloracne. The first indication of chloracne in the workers appeared in January 1933. Following another change in the source of benzene, enclosure of the distillation apparatus and installation of ventilation fans, a gradual improvement in the acneiform eruptions was noted.

In 1936, Schwartz [123] reported digestive disturbances, burning of the eyes, and impotence in men working with chlorobiphenyls. He also noted that nonachlorobiphenyl was used as an insulator for automobile electric wires, in capacitors, and as a delusterer of rayon.

An early use of PCBs was for incorporation along with chloronaphthalenes into synthetic waxes [124-128]. These waxes contained 10-20% PCBs [124-126] and were used to insulate electrical wire and cable. There were several reports that chloracne [125-128] developed in workers involved in the manufacture and use of these waxes, which were associated with at least one fatality in 1936 [125,127].

The fatal case was described by Drinker et al [125] in 1937. The patient, who had been exposed to low concentrations of tetra- and pentachloronaphthalenes (90%) and chlorinated biphenyls (10%) developed chloracne, followed by jaundice. He was hospitalized with abdominal pain and distention. At autopsy, cirrhosis of the liver with superimposed acute yellow atrophy was found. Two other fatal cases were described where the exposures had been to mixtures of penta- and hexachloronaphthalenes [125]. According to the authors, no similar cases had been reported in the literature. As a result of these fatalities, estimates of the airborne concentrations of chlorinated hydrocarbons in 30 different factories were made and animal experiments were performed to study the effects of exposure at such concentrations.

Rats were exposed 16 hours/day, 6 days/week to trichloronaphthalenes at 1.31 mg/cu m, to a mixture of penta- and hexachloronaphthalenes at 1.16 mg/cu m, to a mixture of penta- and hexachloronaphthalenes (90%) and chlorinated biphenyls (10%) at 1.37 mg/cu m, and to a chlorinated biphenyl mixture containing 64% chlorine at 0.57 mg/cu m. The authors [125] stated that higher concentrations had frequently been found in the factories, and that except for trichloronaphthalenes, they did not consider that it would be safe to expose workers to any of the mixtures at the concentrations

studied [125]. Further details of the animal experiment were reported by Bennett et al [129] in 1938. These investigators [129] found morphologic changes in the livers of two groups of rats exposed at the 0.57-mg/cu m concentration and also at 0.93 mg/cu m for 8 hours/day. These animal experiments reported by Drinker et al [125] and by Bennett et al [129] have continued to be erroneously cited [130,131] even though Drinker [132] reported in 1939 that the "chlorinated biphenyl" was actually a mixture of chlorinated biphenyls and chlorinated terphenyls. Drinker [132] stated that a followup inhalation experiment with chlorinated biphenyls containing 68% chlorine showed them to be of low toxicity and he recommended permissible limits for workroom air of 0.5 mg/cu m for mixtures of chlorinated biphenyls and terphenyls and 10 mg/cu m for chlorinated biphenyls.

The first indication in the literature that PCBs might be embryotoxic or have teratogenic effects was the report by McLaughlin et al [133] in 1963, 5 years prior to the recognition of PCBs as an environmental pollutant. The authors evaluated the toxicity of Aroclor 1242 by injecting it into the yolk sac of fertilized eggs prior to incubation, and then observing the effects on embryonic development. None of the eggs hatched after injection with 25 mg of PCBs/egg; with injection of 10 mg/egg, one chick hatched out of 20 injected eggs, but it died 2 days later. Some of the embryos examined showed beak deformities, edema, and retarded growth.

Effects on Humans

(a) Effects from General Environmental Contamination

In the United States, PCBs are present in ambient air [26,37,38],

water [26,37], and in many foods [39,45,46]. A common dietary intake of 10-20 μg/day has been estimated for teenage males in the US [46]. PCBs frequently have been found in various tissues and body fluids of the US population, eg, at ppm concentrations in adipose tissue [7,36,134-136], ppb concentrations in blood [137,138,140,141], and in milk [143,144] at ppm or ppb concentrations in the milk fat or whole milk, respectively.

The Environmental Protection Agency's Human Monitoring Survey has analyzed human adipose tissue samples collected since late 1968 for PCB content [134]. According to this 1972 report, 637 samples had been analyzed, and 198 of these contained more than 1 ppm of PCBs. Positive findings were made in tissues from each of the 18 participating states.

A detailed analysis of two samples collected in the Human Monitoring Survey was made by gas-liquid chromatography (GLC) and mass spectrometry (MS) and reported by Biros et al [135] in 1970. The samples contained at least 14 isomers ranging from penta- to decachlorobiphenyls. Price and Welch [36] stated that of more than 4,000 human adipose tissue samples examined by the Michigan State Department of Health Pesticides, none had chromatograms that exactly matched those of standard Aroclor solutions. Their data show relative accumulation in adipose tissue of the more highly chlorinated compounds, and relative dilution or absence of the less highly chlorinated compounds originally present in Aroclors 1254 and 1260. They [36] described the analyses of tissue samples at autopsy of a 77-year-old man in which PCB concentrations of 100-250 ppm (fat basis) were found The highest PCB concentration was in the liver. The authors [36] found about 55% of adipose tissue samples in the general population contained PCBs at <1.0 ppm, about 36% at 1-2 ppm, and the remainder at more than 2 ppm.

During July 1972 through June 1974, 2,324 fat samples were analyzed by the Human Monitoring Survey, 1,277 in the first year and 1,047 in the second [136]. In the 2 years, respectively, PCBs were not detected in 24.5 and 9.1%, were present at <1 ppm in 40.2 and 50.6%, were present at >2 ppm in 5.5 and 4.9%, and were present at 1-2 ppm in the remainder of the samples. Penta-, hexa-, and heptachlorobiphenyl were the PCBs most frequently present.

A complete analysis of PCB compounds in a composite sample of adipose tissues from patients at the University Hospital in Lund, Sweden, was reported by Jensen and Sundstrom [7] in 1974. Forty-five compounds, accounting for the total PCB content of the adipose tissues, were found and identified by comparison with known PCB isomers. The biphenyl compounds included three tetrachloro isomers, many penta-, hexa-, hepta-, octa-, and nonachloro isomers, and decachlorobiphenyl. By comparison with Clophens A50 and A60, which had compositions similar to Aroclors 1254 and 1260, respectively, the authors [7] found the relative concentration of several compounds with chlorine substitution in the 4,4' positions of the biphenyl ring to have occurred, as did many compounds without vicinal, unsubstituted positions. Most of the compounds which underwent relative dilution to the greatest extent had either vicinal, unsubstituted 3,4-positions or two pairs of vicinal, unsubstituted positions.

Blood sera of 616 residents of urban and rural areas of South Carolina were analyzed for PCBs and the results were presented by Finklea et al [137] in 1972. Analysis was accomplished by GLC with a Ni-63 electron capture detector after basic dehydrochlorination. The amounts of PCBs present were estimated by integration of five peak areas associated

with Aroclors 1254 and 1260. PCBs were not present in samples from all individuals in quantities measurable by the technique used (Table III-3). Analysis of the data indicated that measurable serum PCB concentrations were not related to the age (<5 to >60 years) or sex (305 females, 311 males) of the donor, but that the concentrations associated with race and residence (Table III-3) were statistically different.

TABLE III-3

PCB CONCENTRATIONS IN BLOOD SERUM BY RACE AND RESIDENCE

Race and Residence	No. in Sample	PCBs Measureable In		PCB Concentrations	
		No.	%	Ave* ppb	Max ppb
Rural black	107	5	4.67	9.45	20.6
Urban black	151	57	37.75	5.22	29.0
Rural white	192	119	61.98	5.12	16.6
Urban white	166	89	53.61	4.38	22.0

*Average of measureable concentrations

Adapted from reference 137

Maternal and cord blood samples collected in Tokyo, Japan from December 1973 through February 1974 were reported in 1975 by Akiyama et al [138] to contain PCBs at mean concentrations of 2.8 and 1.1 ppb, respectively (on a whole blood basis). The maximum concentrations found were 7.6 ppb in maternal blood and 3.3 ppb in cord blood. Quantitatively, significant correlations of PCB concentrations in 21 pairs of maternal and cord blood samples were not found. Qualitatively, pairs of maternal and cord bloods had identical PCB patterns resembling those of Kanechlors 500

and 600. The data suggested nonselective in utero transfer of PCB compounds from mother to fetus. Concentrations of PCBs in human embryonic and fetal tissues were reported by Shiota et al [139] in 1973 to not exceed those found in postnatal individuals who died accidentally. The concentrations, found in 19 embryos 5-8 weeks old, were reported as <2 ppb. The concentrations found in 5 second trimester and 2 third trimester fetuses are presented in Table III-4, expressed on the bases of both whole tissue and on the fat content of the tissue.

TABLE III-4

CONCENTRATIONS (ppb) OF PCBs IN TISSUES OF HUMAN FETUSES

Age of Placental Contents	Basis	Tissue Analyzed			
		Cerebrum	Liver	Kidney	Skin
2nd trimester	Whole	2-23	2-33	6-20	17-83
	Fat	150-60	230-800	60-1,900	550-1,300
3rd trimester	Whole	2*	25-90	6-10	48-769
	Fat	270*	1,000-1,300	420-470	880-1,400

*One sample

Adapted from reference 139

PCB concentrations in the venous blood of nine patients hospitalized with severe wasting diseases were reported by Hesselberg and Scherr [140] in 1974. The investigators were concerned with the release of stored organo-halide pesticides and PCBs during mobilization of body fat. They were unable to detect any PCBs in the blood of 15 apparently healthy

control subjects. PCB concentrations found in the patients' blood (uncorrected for efficiency of recovery) ranged from 10 to 100 ppb. Information was not presented on the patients' occupations etc prior to their having become ill.

PCB concentrations in blood plasma and in adipose tissue samples obtained from 28 people during routine abdominal sections had a correlation coefficient of 0.74 according to Inoue et al [141]. The average concentration of PCBs was 6.1±3.52 ppb in blood plasma, and in the adipose tissue (fat basis) it was 2.6±1.9 ppm. These investigators [141] also evaluated the effect of emaciation on PCB concentrations in blood plasma of these and other patients; they found an average of 8.4±4.26 ppb in 19 emaciated patients and 4.7±2.17 ppb in 30 unemaciated patients.

Hair samples collected from a college barber shop were reported by Matthews et al [102] in 1976 to contain PCBs at 0.34-0.76 ppm. The samples were composites from five or more individuals collected on two occasions, 4 months apart. Hair was collected from the barber shop aprons, and care was taken to avoid possible contamination. Five commercial preparations of hair sprays, shampoos, and hair clipper lubricating oil were negative for PCBs. Blood samples were not collected for comparison.

PCBs were reported in 1966 to have been found in hair samples from three members of a Swedish family [142], but no concentration data were given. It was speculated that one of the family members, a 5-year-old girl, had acquired PCBs from her mother's milk.

A correlation was found between the quantities and compositions of PCBs in samples of adipose tissue collected from four women during Caesarean deliveries and in milk samples collected 3-5 days later [143].

There were differences in chromatographic patterns between individuals, but for each individual the chromatographic patterns for the adipose tissue and the milk were qualitatively the same. PCBs in all samples contained principally 4-8 chlorine substitutions. Although the basis (fat or whole milk) for expressing the concentration of PCBs in the milk was not stated, comparisons with PCB concentrations found in adipose tissue and milk by other investigators indicate that the whole milk basis was used [136,144] (EP Savage, written communication, February 1977). Concentration data are summarized in Table III-5.

TABLE III-5

PCBS IN MILK AND ADIPOSE TISSUE OF FOUR WOMEN

Subject	PCB Concentrations, ppm	
	Adipose Tissue	Milk
a	0.62	0.008
b	0.75	0.015
c	1.6	0.032
d	3.1	0.036

Adapted from reference 143

Concentrations of PCBs measured in milk samples from 39 women living in two small cities in Colorado were presented by Savage et al [144] in 1973. Two samples contained PCBs at concentrations of 0.05 and 0.1 ppm. Six other samples contained PCBs at 0.04 ppm, the lower limit of detection for the analytical method, or less. The time postpartum at which the samples were collected and the basis (fat or whole milk) for expressing the concentrations were not mentioned. (Comparison with data in the following

paragraph suggests that the whole milk basis was used.)

Results of analyses of 384 human milk samples from 40 states for PCBs were presented in a written communication by EP Savage in February 1977. All positive samples that contained PCBs at less than 50 ppb on a whole milk basis were recorded as a "trace." Samples with 50 ppb or more of PCBs were reported as ppm in milk fat on the basis of 2.8% milk fat. Only five samples were not positive for PCBs, and 112 samples from 27 states contained measurable amounts (up to 12.6 ppm on a fat basis). Of the total number of samples, 141 were analyzed during December 1976 and January 1977. The time postpartum when the samples were collected was not stated.

While no adverse effects have been associated with PCBs at the concentrations found in adipose tissue, blood, or milk of individuals whose only known exposures were from general environmental contamination [7,36,102,136-144], knowledge of these concentrations is important to the evaluation of reports on occupational exposures. That is, the data provide a basis for evaluating body burdens of PCBs added by occupational exposure and indicate that workers may have substantial body burdens before the added insult of occupational exposure.

(b) Effects from Consuming PCB Contaminated Rice Bran Oil

An episode of poisoning associated with PCB ingestion occurred in Japan in 1968, and was the subject of a special issue of the journal Fukuoka Acta Medica in June 1969 [145-159]. These reports dealt primarily with the situation in Fukuoka prefecture where 325 poisoning cases had been identified through January 20, 1969. The episode resulted from consumption of a particular brand of rice bran oil [160] and ultimately involved persons in 22 prefectures [161]. About equal numbers of cases

(approximately 450 each) were registered in Fukuoka and Nagasaki prefectures as of September 1973 [161]. In the prefectures of Hiroshima, Kochi, and Yamaguchi, there were 80, 45, and 40 cases, respectively, at that time. In each of the other 17 prefectures there were 1-25 registered cases. The total number of cases registered by March 1970 was 1,015; by September 1973, the number had increased to 1,200, and by May 1975 to 1,291 [161,162]. The disease became known as "Yusho," or rice oil disease [145,163]. The outstanding signs of the poisoning were acneiform eruptions and eye discharges (a peculiar secretion from the meibomian glands) [146]. Chloracne was suspected [164]. Hyperpigmentation of the skin, nails, and mucous membranes, swelling of the upper eyelids, and hyperemia of the conjunctivae were other common signs [146,165].

Studies of the rice oils consumed by the patients indicated that the oil associated with Yusho was produced mainly during February 1968 [147,148]. The source of the contamination was determined to be a heat exchange unit containing PCBs that leaked through tiny holes when rice bran oil was heated at low pressure to remove odorous constituents [163,165]. By intensive chemical analyses, including infrared spectrophotometry and GLC, the major contaminant in the rice bran oil was found to be Kanechlor 400 [147]. PCB concentrations in the oil varied, depending on the date of production or shipment. The highest concentration of PCBs, based on the chlorine content of the oil, was about 3,000 ppm which was found in canned oil shipped on February 5. GLC data were not quantitated. In oils shipped thereafter, PCB concentrations decreased rapidly, and only traces were found in oils produced after February 19, 1968 [147,148]. Minor contaminants in the rice oil included polychlorinated dibenzofurans at

about 1/200 of the PCB concentration [20,160], traces of chlorinated naphthalenes, and bromine at about 2% of the chlorine content [147]. Recent analyses of some of the oil samples indicate that there may have been other chlorinated organic contaminants (F Cordle, written communication, November 1976).

In one study [148], the contaminated rice bran oil was found to have been used largely for frying food (which may have altered the constituents). The oil was consumed for various periods during the spring through October 1968 [148]; the first reported clinical examination of a Yusho patient had occurred on June 7, 1968 [163]. Studies through January 1969 of patients in Fukuoka prefecture indicated that onsets of Yusho began as early as February and as late as December 1968, and first involved the eyes [146]. Onset occurred in most patients in June, July, and August [146].

Some attempts were made to estimate the amounts of rice oil and PCBs consumed by the patients [166,167]. Maximum consumption of oil was estimated at 4.4 liters [166]. Isono and Fujiwara [167] estimated that two Yusho cases may have resulted from the ingestion of PCBs at a daily rate of 67 μg/kg body weight for 3 months. For 146 Yusho patients who lived in homes known to have used oil shipped on February 5-6, the estimated average total oil consumption was 800 ml, a volume that contained an estimated 2 g of PCBs [166]. It was estimated that the maximum volume of oil consumed by an individual was 2.7 liters. Of 21 patients who had consumed more than 1,400 ml (3-4 g of PCBs), 18 were considered to have had major signs of Yusho. Among 80 patients estimated to have consumed less than 720 ml of the oil, 31 were thought to have had major signs of Yusho. From the same

data, the minimum PCB ingestion among the 146 patients was estimated at 0.5 g [165]. Estimates of the amount of contaminated oil consumed by 13 pregnant women during the ingestion period ranged from 300 to 2,600 mg [167], but estimates of their PCB consumption were not made. Of the 13 babies born to these women, 8 had jaundice, 3 had marked dermal chromopexy, and 9 had excess secretion of tears [167].

Several studies of PCBs in the tissues and body fluids of Yusho patients were made at various intervals after ingestion of the contaminated oil [146,147]. The samples taken closest to the time of ingestion were collected in October and November 1968 [146,147]. Samples from discharges of the acneiform eruptions of two patients contained PCBs at 32 and 45 ppm, and samples of subcutaneous fat from the face and abdomen of an 18-year-old man contained PCBs at about 75 and 13 ppm, respectively. PCBs with GLC patterns similar to those of the contaminated oil were found in these samples as well as in placental and fetal tissues [147]. Preserved tissues from a baby that had been stillborn in October 1968 were later analyzed and PCB concentrations of 1.8, 1.2, and 0.1 ppm in fat were found in the liver, skin, and fat, respectively [168]. The baby's mother had been classified as a severe case of Yusho with onset about mid-June, but the amount of oil consumed, the period of pregnancy during which it was consumed, and the body burden of the mother were not reported [149]. PCBs were found in all sputum samples from 13 patients collected between December 1969 and May 1970 [169,170]. PCB concentrations were highest in December and detection was less common by May.

Other data on concentrations of PCBs were obtained from body fat and other tissues taken from five Yusho patients at autopsy [168,171]. The

dates of death were between July 1969 and May 1972. Cause of death was heart failure in four cases and a ruptured liver in another case [171]. Estimates of contaminated oil consumed by two of the dead patients were about 0.3 g and 1.6 g, respectively [171]. One PCB, probably a hexa- or heptachlorobiphenyl from the contaminated oil, was especially concentrated in the tissues. The peaks associated with tetrachlorobiphenyls were very low by comparison, suggesting that total PCBs in the body had been substantially decreased within a year after the end of exposure. The mesenteric fat contained PCBs at 0.9-15.1 ppm, and the liver contained 1.3-10.4 ppm in its fat. PCB concentrations in fatty tissues obtained during 11 control autopsies averaged 2.6 ppm. Of the organs examined (liver, heart, kidney, brain, and skin), the liver and heart usually contained the highest concentrations of PCBs [168,171].

In an additional case, the subcutaneous fat obtained on autopsy of a woman who died in September 1972 (about 2 years after consuming contaminated oil) contained PCBs at 2.9 ppm [168].

Polychlorinated dibenzofurans (PCDFs), mainly penta- and hexachloro compounds, were found in tissues obtained on autopsy of two Yusho patients who died in 1969 and of one who died in 1972 [160]. No chlorinated dibenzofurans were found in tissues obtained on autopsy of two controls, although PCB concentrations of about 1-1.5 ppm were found in adipose tissue and liver fat. The findings from the Yusho patients are summarized in Table III-6.

TABLE III-6

PCBs AND PCDFs (ppm) IN FAT FROM THREE YUSHO PATIENTS AT AUTOPSY

Year of Death	PCBs		PCDFs	
	Adipose	Liver	Adipose	Liver
1969	3.4	4.7	0.03	2.3
1969	8.5	5.6	0.04	1.1
1972	2.1	3.5	0.01	0.3

Adapted from reference 160

The ratio of PCBs to PCDFs in the rice bran oil was about 200, as in the adipose tissues obtained at autopsy. However, in the liver, ratios of 2, 5, and 12 indicate that considerable concentration of PCDFs had occurred.

Information on PCBs [146,147,168,171] and PCDFs [160] in tissues indicates some shifts in concentrations during the 3 years after ingestion of the contaminated oil was discontinued. Masuda et al [168] reported that a year after ingestion stopped, the concentrations of tetrachlorobiphenyl components had decreased and were near those found in persons who had not ingested the contaminated oil. The more highly chlorinated PCB compounds were still retained in the fatty tissue 4 years after ingestion had stopped [168].

Blood from Yusho patients was first examined for PCBs 5 years after ingestion of the contaminated oil ceased. Three distinct GLC patterns were found among blood samples from 49 patients [172-175]. Two patterns (A and B) were peculiar to the Yusho patients, and the third pattern (C) was

similar to that of controls. Patterns A and B were characterized by peaks corresponding to those of certain penta- and hexachlorobiphenyl compounds that were present in the contaminated rice bran oil. Patterns A and B differed, however, as to the relative amounts of these compounds [172,173]. These patterns became diagnostic for Yusho [161]. The average concentrations of PCBs in the blood serum were 9, 4, and 2 ppb for patients with patterns A, B, and C, respectively, and 3 ppb for 27 control subjects [173]. The maximum blood serum PCB concentration found among 72 Yusho patients examined between April 1973 and March 1974 was 26 ppb [174]. Of these patients, 43 had pattern A, 26 had pattern B, and 3 had pattern C.

Similar patterns were reported in 1975 by Abe et al [176] in a 1974 study of 18 female Yusho patients and their 30 children. Concentrations of PCBs in blood samples from mothers ranged from 3 to 33 ppb during 1974; PCBs in samples from their children ranged from 1 to 20 ppb, and in samples from 14 control children, from 1 to 8 ppb. PCB concentrations tended to be higher in the blood samples from children nursed by Yusho mothers. A sample of milk from a Yusho mother was reported in 1974 to contain PCBs with a GLC pattern similar to those in samples of fatty tissue from other Yusho patients [168]. Concentrations of PCBs in the mother's milk were 0.06 ppm on postpartum days 0-2, 0.04 ppm on days 3 and 4, and 0.03 ppm on day 5. The respective concentrations in the milk fat were 4.5, 3.0, and 2.6 ppm.

In addition to the skin and eye conditions manifested by most people who consumed the contaminated rice bran oil [146,151-153,162], there was pigmentation of the nails [146] and of the oral mucosa [154]. A substantial array of clinical and laboratory findings were published on

patients with Yusho [150,152,155,156,177-180], including slight increases in activity of serum alkaline phosphatase, reduced serum iron concentrations [155], changes in the microanatomy of liver cells that were considered indicative of microsomal enzyme stimulation [156], symptomatic and functional changes indicative of neuropathy [150,177], respiratory involvement [170,178], a decreased concentration of bilirubin in the serum [179], and, in many patients, an elevation of the concentration of triglycerides in the serum [155,157,158,175,179-181].

Babies born to women with Yusho, both during and after the period of ingestion of the contaminated rice bran oil, were the subject of several investigations [149,159,176,182,183]. Babies of mothers who had ingested the contaminated oil became known as "black" or "cola" babies because of the abnormal skin pigmentation that tended to persist for several months after their birth [176,182,183]. Infants born up to 5 years after their mothers' last ingestion of contaminated rice oil were still affected to some extent [182]. In one study of four babies, other clinical and anatomical abnormalities (retarded intrauterine growth in three, edematous face and exophthalmic eyes in three, dentition in two, calcification on skull and wide, open sagital suture of skull in three) were seen at birth [183]. These were not permanent, and postnatal body and mental development appeared normal in these and other Yusho children [160,176,183]. In at least one case, an investigator concluded that a baby had developed Yusho from nursing [182].

The period of ingestion of contaminated rice bran oil was only a few months, but the effects have persisted for several years [160,161,174,175,179,180,184,185]. The skin lesions and hypersecretion

from meibomian glands remained unchanged for long periods in many patients [174,184,185]; their magnitudes in 1974 appeared to be related to blood PCB concentrations [174]. In addition, complaints of generalized fatigue, and symptoms referable to the peripheral nervous system and to the respiratory system became more prominent. In 1974 these complaints and symptoms were established as part of the diagnostic criteria for Yusho [161]. These additional symptoms were not related to the PCB concentrations in the blood [174].

Elevated serum triglyceride concentrations were found to be related to the concentration of PCBs in the blood serum [175], and inversely related to the bilirubin concentrations in the serum [179]. The average serum triglyceride concentrations measured annually in 14 Yusho males from 1969 through 1974 ranged from 159 mg/100 ml in 1969, to 174 mg/100 ml in 1972, to 160 mg/100 ml in 1974 without any significant changes from year to year. In 29 Yusho females, the concentrations initially (1964) averaged 153-161 mg/100 ml, but decreased in 1973 and 1974 to 129 and 111 mg/100 ml, respectively [180].

The Yusho population of about 1,300 persons has been followed closely and records indicate that as of May 1975, 29 of them had died [162]. Deaths have been due to a variety of causes such as accidents, suicides, cardiac problems, and cancers. Malignant neoplasms were found in at least nine cases [161], from which Kuratsune et al [160] concluded that there was a suggestion of excess deaths but that no more could be said because information essential for analysis was not available.

The relevance of the Yusho episode to occupational PCB exposure is compromised because: (1) the oil was ingested; and (2) it contained large

concentrations of dibenzofurans compared with those in the PCBs to which workers generally have been exposed in their occupations. Its relevance is further compromised because the effects observed from daily ingestion of 1-15 mg of PCBs [165,167] were peculiar and excessive compared to those observed in workers exposed by inhalation to PCBs at 1-5 mg/cu m [120,125], notwithstanding that the amounts absorbed may have been similar.

Nevertheless, information obtained from the Yusho episode is relevant to the study of PCB toxicology and occupational exposure. The information establishes that PCBs can be transmitted from mother to fetus, and, in the milk, from mother to child. It also establishes that some PCB compounds are eliminated from the body relatively rapidly, and that others may require years for elimination.

(c) Occupational Exposures

Chloracne was among the earliest reported effects associated with worker exposure to PCBs [125-128]. It was not clear in some early reports that PCBs were contributing to the chloracne because PCBs usually constituted 10-20% of mixtures containing 80-90% chloronaphthalenes, a previously known cause of chloracne [121,122]. An early report that associated chloracne with PCBs in the absence of chloronaphthalenes was that of Jones and Alden [120] in 1936. The manufacturing process was largely an open one and the workers were also exposed to benzene, biphenyl, and other compounds incidental to PCB manufacture. Over the years, cases of chloracne associated with occupational exposure to PCBs have continued to appear [186-196].

Other effects associated with PCBs in early reports included digestive disturbances, eye irritation, liver injury, and impotence

[123,125]. Elkins [130] reported in 1950 that the average concentrations of PCBs in the workroom air of several plants in Massachusetts ranged from 0.1 to 5.8 mg/cu m. Maximum concentrations ranged from 0.2 to 10.5 mg/cu m. No evidence of immediate toxic effects was observed except at PCB concentrations approaching 10 mg/cu m, which the workers found to be unbearably irritating.

Three cases of severe chloracne were described by Puccinelli [186] in 1954. The affected employees worked in a factory that produced capacitors impregnated with Aroclor 1254. The workroom was 24 x 9 x 5 meters. Capacitors were heated to about 100 C in a 3-cu m autoclave to remove moisture, and then impregnated with the Aroclor while in the autoclave. The temperature in the autoclave was reduced about 12 hours later. When the autoclave was opened, the temperature of the Aroclor was 70-80 C. The capacitors were removed and carried to another location for finishing. Originally, one autoclave was used, but eventually eight were in operation, and PCB emissions to the workroom air occurred almost continuously. Concentrations of PCBs in 500-liter samples of air were found to vary from 5.2 mg/cu m in the center of the room, to 6.4 mg/cu m around the finishing operation, to 6.8 mg/cu m near the autoclave during removal of the capacitors. The three chloracne cases were in men, 18-24 years of age. They had worked in the factory for 2-4 years, and developed the first signs of chloracne 4-8 months after their exposures began. Other than chloracne, the men appeared healthy, and all findings, including liver function tests were reported as normal.

Eight other cases of chloracne associated with PCB exposure during the manufacture of radio capacitors were described by Hofmann and Meneghini

[187] in 1962. In the process, PCB vapors were generated by heat. The cases included one man and seven women, 20-37 years of age, who were exposed to PCBs 2.5-4 months before onset of signs of chloracne. The face was involved in six cases; other involved areas varied, but included the arms, neck, upper torso, pubes, buttocks, and thighs. The case of a 21-year-old woman was described in detail. The first signs of chloracne appeared on her face 4 months after she began work, and she was observed by the authors [187] 4 months later. On the face, a dirty brown coloring appeared. A hyperpigmented spot with shaded areas was present on the forehead. Subsequently, her buttocks and pubes became involved. Exposure concentrations were not mentioned.

Severe chloracne was described by Birmingham [188] in 1964 in 13 of 15 workers exposed to an Aroclor which was a mixture of bi- and terphenyls (65% chlorine content). An enamel containing the Aroclor was painted onto glass and then baked in an oven. Faulty ventilation caused contamination by chlorinated hydrocarbon vapors. Exposure concentrations and duration of exposure were not given.

An additional case history published in 1969 involved a 43-year-old man exposed to PCBs in an electrical component factory [189]. He developed chloracne on the forehead, face, arms, and thighs within 3 months after beginning work handling racks of electrical parts that had been dipped in hot PCBs. Exposure concentrations and actual durations of exposure were not stated, but it was reported that the man had put his hands in the mixture without skin protection for a long time, and that his clothes often became impregnated with the PCBs. On examination 8 months after transfer to another job in the same room, papules, comedones, and pustules were

found on his forehead, scalp, face, and arms. Although the man had been removed from direct exposure to PCBs, their odor was present in the man's new work area. It was reported that this was the first case of chloracne in the plant, where more than 100 workers had been engaged in the process for more than 20 years.

A company that used PCBs at two manufacturing facilities provided testimony on employee health in 1975 (In the Matter of General Electric Company, File No. 2833, New York State Department of Environmental Conservation). Examination of records submitted as testimony indicated that exposures were to an askarel [25] containing, by weight, about 60% of Aroclor 1254, 40% trichlorobenzene, and 0.0115-0.135% diepoxide scavengers. From the records submitted, it was not possible to determine the precise numbers of male and female workers. The exact occupations of the workers could not be determined either but a substantial number of "crimpers" was indicated.

The records showed that employees had reported to the dispensary complaining of skin rashes and dermatitis on 49 occasions during the previous 15 years. Only the fingers and hands were involved in 21 complaints, only the arms and hands in 5, and only the face and legs in 1. In 7 other complaints the face, neck, and legs were mentioned in 4, 2, and 1 cases, respectively, in addition to the upper extremities. Associated with the complaints of skin rashes and dermatitis were some complaints of itching of the face (2), neck (1), eyes (1), arms (2), and hands (1). A generalized skin rash was the basis of 14 additional complaints. One worker developed the generalized rash on exposed parts of his body after only 2 days of working with the askarel.

The company physicians attributed the rashes to allergic or contact dermatitis caused by exposure to the askarel. Treatment included the use of creams, and temporary or permanent removal from exposure. Of the 49 complaints, 22 were second episodes, and in these cases the workers were removed permanently from exposure to askarels. One female employee who had dermatitis of the fingers was removed from exposure for 12 days. Within 2 days of reexposure she again reacted to the askarel and was permanently removed from such work.

Over the same 15 years, other kinds of complaints were made by the workers on 16 occasions. These complaints included burning sensations of the eyes (7), nose (1), and face (1); dry throat (1); asthmatic bronchitis (3); nausea (1); dizziness (1); and aggravation of acne (1). In most of these cases, the company physicians recommended permanent removal from exposure.

The health status of eight laboratory workers who routinely analyzed dielectric fluids containing PCBs was reported by Levy et al [197] in 1977. The men were 25-49 years of age and had been employed 2.5-18 years. Breathing zone, point source, and general work area air samples were collected on magnesium silicate at 50 ml/minute over the workday on three occasions. The breathing zone samples contained PCBs at 0.014-0.073 mg/cu m. Samples taken near an oven contained 0.042-0.264 mg/cu m, and general room area samples contained PCBs at 0.013-0.15 mg/cu m. The blood PCB concentrations in the workers were 36-286 ppb. The most common complaint of the workers (6 of 8) was dry or sore throat. Other complaints were skin rash (3 of 8), gastrointestinal disturbances (3 of 8), eye irritation and headache (each, 2 of 8). Findings on examination of the eight workers were

skin rash (1), nasal irritation (2), rales (1), and elevated blood pressure (4). No liver, spleen or neurologic abnormalities were found by physical examination. There were no cases of chloracne. Serum alkaline phosphatase, SGOT, SGPT, and total bilirubin, measured in seven subjects, were all within normal limits. In addition, medical records of 40 other exposed employees were examined, revealing two cases of slightly increased SGOT, two elevated serum triglycerides, and one case each of increased serum alkaline phosphatase (SAP) activity, total serum bilirubin, and serum uric acid.

Epidemiologic Studies

An outbreak of dermatitis among workers in a Connnecticut chemical plant was described in 1954 by Meigs et al [190]. PCBs had been substituted for molten salt in a heat exchange unit without modification of the system. There were slight, but obvious, vapor leaks under certain conditions, and the concentration of PCBs in the workers' breathing zones was determined to be 0.1 mg/cu m. No employee worked regularly at points of leakage, and the operations, as described, continued for 19 months.

Mild to moderate chloracne on the face, forehead, and ears developed in 7 of 14 exposed workers; the mastoid region of one worker also was affected. The duration of exposure before the initial signs occurred ranged upward from 5 months and averaged 14 months. The average length of exposure was 11 months for those who did not develop chloracne, with one worker showing no signs after 19 months. Liver function tests were performed on the seven workers who had developed chloracne. Clinical tests included direct and total serum bilirubins, 24- and 48-hour cephalin

flocculations, thymol turbidity, and SAP activity. Findings were normal in six workers, and borderline increases in cephalin flocculation and thymol turbidity were found in the seventh worker with chloracne. Thirteen months later, the thymol turbidity test had improved, but cephalin flocculation had not changed. All cases of chloracne were stated to have cleared up after an unspecified treatment. Control of vapor emissions by welding all joints in the heat exchange unit prevented recurrence [190].

Exposures of workers to PCBs in six industrial plants were discussed by Hasegawa et al [191] in 1972. The concentrations of PCBs found are summarized in Table III-7.

TABLE III-7

RANGE OF PCB CONCENTRATIONS (μg/cu m) IN WORKROOM AIR

Factory	Function	No. Samples	PCB Concentrations Vapors	Particulates
A	PCB manufacture	6	26-163	19-37
B	Capacitor manufacture	3	120-350	20-125
C	Biphenyl recovery	2	13-15	4*
D	Capacitor manufacture	2	350-540	48-6,270**
E	"	3	95-965	73-650

*1 sample
**6,270 due to spillage

Adapted from reference 191

PCBs were manufactured in one plant and used in manufacturing capacitors in four plants (one had discontinued use of PCBs 1 month earlier). Biphenyl, not chlorobiphenyls, was present in the sixth plant [191]. Air samples were collected in two fractions. A fraction associated with particles >0.1μ was collected on filter paper, and a fraction containing vapors and particles <0.1μ in diameter was collected in two serially-connected midget impingers containing n-hexane. Samples were collected only from places where high concentrations of PCBs were expected and not from the factory where PCB use had been discontinued.

The vapor concentration exceeded the particulate concentration in all except the sample taken after spillage. Particulate matter was characterized by GLC as containing the same chlorobiphenyl composition as the PCB product used in the plant, whereas the vaporized material contained one less chlorine atom/molecule than the PCB used in the plant.

PCBs were measured in the blood of the employees of six plants, including 99 exposed workers and 32 controls [191]. Concentrations of PCBs in the blood sera of exposed workers averaged 370 ppb, whereas those of the controls averaged 20 ppb. The workers exposed to PCBs in the plant that had discontinued PCB use had serum PCB concentrations of 90 to 730 ppb (average 460 ppb). Based on data from three of the plants, no relationship was found between duration of exposure (from <1 to 20 years) and concentration of PCBs in the blood [191].

Complaints of dermal ailments seemed to be unrelated to the blood PCB concentrations and were considered to be due more to direct contact of the skin with PCBs than to generalized intoxication. The principal dermal findings included brown chromodermatosis of the dorsal joints of the hands

and fingers and of the nail bed, and acneiform exanthema. The latter also involved the jaw, back, and theighs in several cases. The investigators [191] considered that there was a definite effect of PCBs on fat metabolism, as shown by decreases in the concentrations of total, free, and esterified cholesterols in the blood, and by trends in the same direction for neutral fats, total glycerides, phospholipids, and beta-lipoprotein in the blood. There was evidence of mild disturbances of liver function manifested by increased SGOT, SGPT, and SAP activities and decreased activity of serum cholinesterase. These enzyme activity changes were not considered to be clinically significant.

Studies of 38 current and 80 former employees of a capacitor factory in which Kanechlor 500 had been used from 1954 to about 1960 and Kanechlor 300 had been used from about 1960 to 1972, with PCB use having been discontinued in April 1972, were reported by Hara et al in 1973 [192] and again in 1974 [193]. (Presumably, this factory was the one stated by Hasegawa et al [191] to have discontinued use of PCBs.) Current workers included 17 who were engaged in the capacitor immersion process; the remaining 21 workers were engaged in finishing and assembling operations. The study concentrated on the 17 immersion process workers. During exposure to PCBs in March 1972, the concentrations of PCBs in the whole blood of the immersion workers ranged from about 7 to 300 ppb, and were closely related to years of exposure. One year later, blood PCB concentrations had all decreased, but by varying amounts. For example, one of two workers who initially had PCB concentrations in their blood of about 180 ppb had a concentration of <10 ppb 1 year later, while the other one had a concentration >100 ppb. The average PCB blood concentration for the

17 workers decreased to about 75% of the original value. Based on blood samples collected about 6 and 12 months after use of PCBs was discontinued, the blood PCB half-lives for the immersion workers were calculated. It was found that the greater the duration of exposure, the greater the PCB half-life (1 year of exposure, 3 months half-life; 10-15 years of exposure, 30 months half-life). This indicated to the investigators that blood served as a PCB carrier, whereas fat served as a depot tissue [192].

While working with PCBs, many of the total group of employees had dermal complaints (blackheads, 45%; acneiform eruptions, 37%; skin irritations, 13%) [192]. A year after discontinuance of exposure to PCBs, these conditions had improved noticeably and only one or two blackheads remained. No correlation was apparent when concentrations of PCBs and triglycerides in the sera of a large number of workers were compared graphically. The workers were also studied about 18, 24, and 36 months after use of PCBs had been discontinued [193]. During this period, blood PCB concentrations decreased to about 10-20 ppb in all but two workers who had been exposed to PCBs for 9 and 15 years. The skin disturbances were reduced to vestigial markings on a few individuals. Comparisons with the concentrations of triglycerides and PCBs in the sera of these workers indicated that the proportion of workers with significant increases in triglyceride concentrations increased as the concentrations of PCBs increased. Of nine workers with blood PCB concentrations >50 ppb, five had elevated triglyceride concentrations.

Examinations of 13 workers from an electrical capacitor manufacturing plant for clinical manifestations of PCB toxicity were reported by Kitamura et al [194] in 1973. Examinations were performed when the company

discontinued the use of PCBs in June 1972, and were performed twice subsequently at 3-month intervals. The average length of worker exposure to PCBs had been about 2.5 years. Immediately after discontinuance of PCB use, the average PCB concentration in the blood of workers was 820 ppb, ranging from 320 to 2,100 ppb [194]. The mean concentration fell to 310 ppb after 3 months, and to 200 ppb after 6 months. From these observations, Kitamura et al [194] estimated the biological half-life of the PCBs in the blood immediately following the cessation of exposure to be about 90 days. No consistent correlation could be found between the concentration of PCBs in the blood and the duration of exposure to PCBs. Nail, hair, and gum color and color of the mucous membranes of the oral cavity all were normal. Ten of the workers had varying degrees of skin disorders on different parts of their bodies. The skin disorders included seborrhea adiposa, acne vulgaris, and follicular papules on parts of the body where direct contact with the PCBs normally did not occur. The authors [194] concluded that PCBs probably had been an important factor in the etiology of the skin disorders and that results of blood tests, hepatic function tests, and urinalyses were normal. Serum triglycerides were not determined.

A study by Inoue et al [195] of the health of workers in family-owned silk-thread glossing factories in which PCBs were used was published in 1975. The study was initiated because PCB concentrations exceeding 100 ppb had been found in the blood of a 73-year-old man who had undergone surgery. His family operated a household thread-glossing factory. The family members and the hired helper were studied. Serum concentrations of PCBs resembling Kanechlor 500 ranged from 130 to 520 ppb. There was a close

correlation between the PCB concentration in the blood and the degree of involvement in the glossing work. The head of the household had skin lesions and comedones on the face, back, and ears and had blood PCB concentrations of 190-210 ppb. The rest of the people had practically no skin abnormalities, and other findings (not described) were, in general, considered of minor significance. A study of PCB contamination of the premises and the air of the same factory was reported by Fujiwara et al [33]. At the time the samples were taken, use of PCBs had been discontinued. However, PCBs were found in air at 0.25 mg/cu m, in floor boards at 80-130 ppm, in the dirt under the machinery at 10-900 ppm, and in the dust on steel frame beams at 110-180 ppm. The PCBs were similar to those in Kanechlor 500.

Subsequent to the above study, Inoue et al [195] reported on the PCB concentrations in samples of blood obtained from 54 other similarly employed people. PCB concentrations of over 100 ppb, 50-99 ppb, 10-50 ppb, and 0-9 ppb were found in 2, 5, 19, and 28 persons, respectively. Correlative comparisons with the various functions performed in the factories showed that those workers who had direct contact with the glossing machines, those who maintained and repaired the machines, and those who had over 20 years of work experience had the highest concentrations of PCBs in their blood. Skin abnormalities and other findings in these 54 persons were described as relatively mild. One of the persons studied, a woman in her 10th month of pregnancy, had a serum PCB concentration of 24 ppb. Following birth, the mother and daughter were described as healthy with no evidence of abnormalities. The concentration of PCBs in the mothers's milk was found to be 0.25 ppm, and after

consultation, she stopped nursing her baby [195].

In 1974, Sato and Hasegawa [32] discussed their findings on PCB residues in the workers and in the air of pressure-sensitive ("carbonless") copying paper manufacturing plants 2 years after PCB use had been discontinued. A PCB product comparable to Kanechlor 300 was still detected in the workroom air of four of five factories at concentrations ranging from 0.13 to 4.4 μg/cu m; in one factory PCBs comparable to a mixture of Kanechlors 300, 400, and 500 were found at concentrations ranging from 0.15 to 1.2 μg/cu m. Concentrations of PCBs in the blood sera of these workers, as measured by GLC, ranged up to 73 ppb, compared to a maximum of about 20 ppb in controls. The authors [32] concluded that the blood PCB concentrations in the workers were still elevated.

PCB concentrations in the blood of three groups of employees with different PCB exposure histories were presented in 1972 by Karppanen and Kolho [198]. The first group of four men and five women had no known occupational exposure to PCBs. The six women of the second group had been exposed while handling PCBs in an analytical laboratory. The third group of eight men and four women had worked, since 1968, in a plant where Aroclor 1242 was impregnated into capacitors. The authors [198] stated that the workroom air of the capacitor factory met internationally accepted limits (presumably <1 mg/cu m), and that protection of the skin had been given special attention. PCB measurements were made by GLC, using electron capture detection. The unexposed group had blood PCB concentrations of 5.6-12 ppb, the analytical laboratory workers had concentrations of 36-63 ppb, and the capacitor plant workers had concentrations of 74-1,900 ppb. Subcutaneous adipose tissue samples from two of the unexposed workers

contained PCBs at 1.5 and 2.3 ppm; PCBs in their blood expressed as concentrations in fat of the blood were 6.6 and 9.9 ppm, respectively. In three capacitor plant workers, adipose tissue samples contained PCBs at 160, 285, and 635 ppm; PCBs in their blood, expressed as concentrations in their blood fat, were 400, 305, and 700 ppm, respectively. All persons examined were in good health. The capacitor plant workers had been under special medical observation but the investigators were unable to detect any biologic effect from the PCBs.

An epidemiologic investigation of 37 refuse workers who were potentially exposed to PCBs emitted from incinerated waste was described by Bumgarner et al [199] in 1973. The control group consisted of 36 workers from a lumber yard. Paired samples of scalp hair and blood were collected. PCB residues in the hair and blood plasma were extracted and analyzed by GLC with electron capture (EC) detection; rough quantitation was by evaluation of five peaks associated with Aroclors 1254 and 1260. The lower limit of detection was 1 ppb. Hematocrit, blood cholesterol, and blood pressure also were determined. PCB residues in the blood plasmas of the controls were detected in four workers and the maximum concentration was 4.2 ppb. Measurable concentrations of PCBs were found in the blood samples of 32 of the 37 refuse workers; the average and maximum concentrations were about 4 and 14 ppb, respectively. The concentrations of PCBs in plasma were not related to duration of exposure, age, or race. PCBs were not detected in hair samples (limit of detection, 1 ppb). Hematocrit, blood cholesterol, and blood pressure values did not change at different PCB concentrations.

A survey of the health of 34 workers exposed to Aroclor 1242 during manufacture of capacitors was discussed by Ouw et al [196] in 1976. The Aroclor was an electrical-grade material that contained "no impurities." Breathing zone samples were collected in impingers containing isopropanol. It is not clear if all samples were collected from the breathing zones. Nineteen workers were assigned to fill capacitors with Aroclor 1242 heated to 70 C. These workers were exposed to PCBs at 1.08-1.44 mg/cu m. The other 15 workers, located in a different room, were assigned to assemble Aroclor-dipped capacitor components. These workers were exposed to PCBs at 0.32 mg/cu m. None of the 34 workers used protective clothing.

PCBs in the blood were separated by GLC and identified by their retention times relative to aldrin. PCBs were not detected in the blood of 30 control subjects. For the two groups of PCB-exposed workers, averages of measurable blood PCB concentrations are presented in Table III-8.

TABLE III-8

AVERAGE OF DETECTABLE BLOOD PCB CONCENTRATIONS (ppb)
OF TWO GROUPS OF WORKERS

Exposure Group	No. Workers	PCB Retention Time Relative to Aldrin			
		0.69	1.31	1.47	1.96
Fillers	19	602(19)*	314(17)	391(13)	475(4)
Assemblers	15	140(14)	100(14)	899(5)	(0)

*Numbers in parentheses are the numbers of workers in whom more than a trace of the PCB peak was detected.

Adapted from reference 196

Distribution of PCBs in the blood of workers exposed while filling the capacitors differed from that of workers who assembled the components. The higher boiling components were present more frequently and to a greater extent in the fillers.

Twelve of the workers (5 of 19, and 7 of 15) complained of mild burning and irritation of the face, eyes, and skin, and 5 of these had eczematous rashes on the hands and legs. One filler had chloracne. Although individual abnormalities were found in SGPT, SAP, and bilirubin, the average values for the exposed workers were within normal limits. Bromsulphothalein retention tests were found to be elevated in four of the seven fillers whose blood PCB concentrations were >500 ppb. The investigators [196] reported no evidence of significant adverse responses to PCB exposure in the workers with blood PCB concentrations below 200 ppb.

Subsequently, "more efficient" exhaust ventilation was installed and the workers were advised to wear "suitable impervious gloves" [196]. Air measurements, made after the ventilation change, indicated that the fillers were being exposed to PCBs at 0.18-0.75 mg/cu m and the assemblers at 0.08 mg/cu m. These were substantial reductions. However, blood PCB concentrations that were found in 15 workers reexamined 2 months after the ventilation change did not show any substantial reductions. The investigators [196] speculated that this might have been because the workers did not strictly follow the recommendation to wear protective clothing.

Based on a search of chart records, Bahn (HA Sinclaire, written communication, June 1976) reported a preliminary study of the incidence of cancer in a group of 51 research and development employees and 41 refinery

plant employees at a New Jersey petrochemical facility who were considered likely to have been exposed to Aroclor 1254 for various periods between 1949 and 1957. The eight cancers observed in the study population through December 31, 1975 were not significantly more than would be expected (5.7) in a similar sample of the US population [200]. However of the eight cancers, the three melanomas and the two cancers of the pancreas were significantly different from calculated expectations. Some findings of this preliminary investigation were described in a letter from Bahn et al [201] to the editor of the New England Journal of Medicine.

In this preliminary study, PCB exposure histories were based on recollections of two company employees. Exposures to other chemicals could not be ascertained. The expected cancer rates were based on US population data rather than on a rate for the locality of the petrochemical facility. To correct these deficiencies in the preliminary study, a more intensive investigation is being conducted (BN Kightlinger, written communication, November 1976). A substantial change has occurred in the cohort since release of the preliminary report by Bahn and her coworkers, and it seems likely that the findings on this new cohort will differ significantly from those of the preliminary study. The final report is not yet available.

In a study of current and former employees engaged in the manufacture of PCBs, no cases of malignant melanoma or pancreatic cancer were found from a review of the case histories of more than 300 employees (G Roush, written communication, September 1976). Seven cases of lung cancer were found from the death certificates of 50 former employees compared to an expected number of 2.7. The data are preliminary and were not corrected for age or smoking habits. The final report is not yet available.

Animal Toxicity

Although there have been a few reports of dermal and inhalation experiments with animals, most of the information on animal toxicity has involved ingestion of PCBs in the diet or by intubation. Several animal experiments have involved oral administration of PCBs for the lifetime of the animal. These studies have demonstrated chronic changes in the microscopic anatomy of the liver and other organs, effects on reproduction, embryonic and fetal toxicity, effects on offspring from nursing, and carcinogenic and teratogenic responses.

(a) Inhalation and Dermal Application Studies of PCB Mixtures

Absorption and distribution of inhaled PCBs were studied by Benthe et al [97] in 1972. Groups of 4-6 male Wistar rats were exposed to aerosols of a commercial PCB mixture containing 42% chlorine, Pydraul A 200, commonly used in hydraulic fluids. Absorption and distribution of the PCBs were studied through the measurement of PCB concentrations in liver, brain, and adipose tissue. The aerosols were produced in an aerosol generator at 180 C. The aerosols were cooled, the larger particles were separated out, and the airborne PCBs were introduced into a chamber at a concentration of 30.4 ± 3.4 g/cu m. Groups of the rats were exposed for varying periods up to 2 hours. It was found that 15 minutes of exposure was sufficient to attain more than 50% of the PCB concentration of 69.7 μg/g liver wet weight that could be attained with 2 hours of exposure. Consequently, the investigators used an exposure time of 30 minutes to study distribution of PCBs to the tissues.

Immediately after 30 minutes of exposure, PCB concentrations were maximal, at about 52 μg/g liver tissue, whereas they were at low

concentrations in the other tissues at 14 μg/g in adipose tissue, and 9 μg/g in brain tissue. After 24 hours the PCB level in the brain attained a maximum value of about 18 μg/g and within 36 hours the PCB concentration in the adipose tissue attained a maximum of about 250 μg/g. By 48 hours the concentrations of PCBs in the liver were reduced to about 3 μg/g and in the brain to about 5 μg/g, whereas the adipose tissue concentrations remained above 200 μg/g.

Experiments were reported by Rozanova [202] in 1943 in which rats were exposed to a technical mixture of "tetrachlorodiphenyl" and "pentachlorodiphenyl." The mixture, known as Solvol, was a transparent, colorless, oily, very viscous liquid used for filling capacitors. The "tetrachlorodiphenyl" component of the mixture had a distillation range of 220 to 245 C and the "pentachlorodiphenyl" component had a distillation range of 242 to 260 C. The animals were exposed in a 22-liter chamber through which air was drawn continually at a rate of 1-1.5 liters/minute, after first passing through a glass gooseneck containing the liquid PCBs. Air samples were taken from the chamber from time to time to determine the PCB concentrations. The analytical method was not reported.

Four rats exposed for 3 hours at about 10 mg/liter became uncoordinated and comatose and died within a day [202]. Autopsy findings included liver necrosis and fatty degeneration, cloudy swelling of the epithelial cells of the renal tubules, congestion in the heart and spleen, and necrotic signs in the spleen. Three rats were exposed repeatedly to vapors of Solvol at 0.5 mg/liter. One rat died after eight exposures, and the other two were killed after 11 exposures. Five other rats were repeatedly exposed at 0.25 mg/liter; one was killed after 16 exposures, and

the others, which appeared to be in satisfactory condition, were killed after 69 exposures. In these chronically exposed rats, gross and microscopic findings were similar to, but less marked than, those found in the acutely exposed animals. In addition, hyperplasia of the Kupffer cells was found in the liver.

Treon et al [203] exposed groups of animals each comprised of 10 rats, 10 mice, 6 guinea pigs, 4 rabbits, and a cat to Aroclor 1242 or Aroclor 1254 vapors 7 hours/day, 5 days/week for up to 31 weeks. Aroclor 1242 exposures at concentrations of 8.6 μg/liter (0.83 ppm) for 3 weeks, 6.83 μg/liter (0.66 ppm) for 17 weeks, or 1.9 μg/liter (0.18 ppm) for 31 weeks. Exposures to Aroclor 1254 were at 1.5 μg/liter (0.11 ppm) for 31 weeks or at 5.40 μg/liter (0.41 ppm) for 17 weeks. No consistent changes in mortality, growth, pathology, organ size, liver function, or hematologic parameters were found in animals exposed to Aroclor 1242. The animals exposed to Aroclor 1254 vapors showed no changes in growth or mortality but microscopic evidence of apparently reversible hepatic cellular injury was found in all species except the cat at both exposure levels. Enlarged livers were found in the animals exposed at 5.40 μg/liter. An appreciable incidence of pneumonia was found among the exposed and control animals, a fact which could have confounded some of the results, but it is important to note that liver changes, including fatty degeneration, were found in exposed animals that were free of pneumonia.

Inhalation experiments with the commercial PCB product "Decachlorodiphenyl" were reported by Berczy et al [98,99] in 1974. The oral toxicity of this PCB product was investigated by Hunter et al [204], who administered it in the diet to Sprague-Dawley rats at 1,000, 2,000,

5,000, and 10,000 ppm for 4 weeks. All animals fed Decachlorodiphenyl gained more weight than the controls and had greater liver to body weight ratios. Other effects observed at 10,000 ppm included increased spleen, thyroid, and kidney weights relative to body weights, reduced hemoglobin concentrations, and in males reduced rbc counts and hematocrits.

In one inhalation experiment [98], five male and five female rats were exposed for 6 hours to particles of the PCB product at an average concentration of 2.54 mg/liter. Seventy-eight percent of the particles were in the range of 1-5 μm in diameter, 17% in the range of 5-15 μm, and the remaining 5% were >15 μm. During exposure there were repeated episodes of blinking and sneezing. Signs of irritation disappeared after cessation of exposure. During the subsequent 14-day observation period, food and water consumption and growth of the rats were considered by the authors to have been similar to those of the controls. No gross pathological changes were seen when the rats were killed 14 days after removal from exposure [98].

Subsequently [99], three groups of rats, each consisting of 8 males and 8 females, were exposed 6 hours/day, 5 days/week for 4 weeks at average concentrations of 4, 80, and 777 μg/liter. A similar group of rats was used as a control. In this experiment, where 85-90% of the particles were in the 1-5 μm range and 1-3% were >15 μm, no signs of irritation were seen during the exposures at the lower concentrations and growth rates were normal compared to controls. At the highest concentrations, frequent blinking and sneezing were noted during the exposures, and the growth rate of the males was slightly retarded (final body weights of 395 g vs 435 g for controls). The liver weights, relative to body weight, were increased

in males exposed at the highest concentration, and in females exposed at the medium and high concentrations. Microscopic findings in the livers of the rats exposed at the high concentration included occasional focal aggregations of mononuclear cells with either parenchymal or periportal distribution, and minimal degrees of periportal hepatocytic vacuolation, and decreased centrilobular or periportal glycogen. These findings were not considered by the authors [99] to be of toxicologic significance. A statistically significant decrease in packed cell and mean cell volumes was found in male rats exposed at the high concentration. In this group there was also a low white cell count due to a decrease in the number of lymphocytes, and increased thrombocyte activity. Blood glucose, SGOT, and serum sodium concentrations were decreased.

Von Wedel et al [205] described in 1942 the results of an experiment performed to determine the systemic effects of exposure of mice, guinea pigs, and rabbits, by inhalation, ingestion, and dermal application to an unspecified Aroclor. Concentrations and durations of exposure were not specified for the inhalation and ingestion experiments. However, 0.5, 1.0, or 1.5 ml of solutions containing 0.5 g Aroclor/ml were used for the dermal applications. Within 5 days after the dermal applications, small papules and blisters formed on the exposed skin areas and the external epidermal layers became desquamated. In addition, subacute yellow atrophy of the liver with some fatty infiltration was observed. Similar liver lesions were produced in the inhalation experiment.

In 1944, Miller [206] administered Aroclor 1242 to rats, rabbits, and guinea pigs by subcutaneous (sc) injection, by oral intubation, and by dermal and corneal application. The PCB doses ranged from single doses of

69 mg to small drops (approximately 17 mg) applied daily to the cornea of rats for 25 days, to 1,380 mg injected daily into rabbits for 10 days. Fatty degeneration and atrophy of the centrilobular cells of the liver were the characteristic signs of toxicity. The greatest amounts of liver damage were seen in guinea pigs, less was seen in rabbits, and the least amount in rats, regardless of the dose, duration of exposure, or route of administration. Necrotic lesions were also seen in the skin of animals that received sc injections; signs of dermal irritation were seen after applications to the skin. The conjunctival tissue presented no gross changes when examined under magnification in the living animals. Since the pathologic changes in internal organs were similar regardless of method of administration, this experiment indirectly demonstrated that PCBs could be absorbed through the skin and the eye.

The effects of Solvol applied to the ears of rabbits were reported by Paribok [207] in 1954. Solvol was applied for 6 hours daily in doses of 0.7 to 3.76 g. A single application caused edema and inflammation of the ear, and one rabbit died 7 days after the application. With multiple doses, the animals died after 6-17 days. The dead animals, including the one that died after the single application, had fatty degeneration of the livers.

Vos and Beems [208] reported in 1971 on the dermal toxicity of PCBs in adult female New Zealand rabbits. Three commercial preparations of PCBs were used: Clophen A60, Phenoclor DP6, and Aroclor 1260. Twenty-seven 1-ml (118 mg) applications of each of these products (in isopropanol), 5 times/week over 38 days, to the clipped and shaved backs of the rabbits resulted in various manifestations of toxicity.

In general, the toxic signs were most pronounced in the Clophen-treated animals and least pronounced with Aroclor 1260. Dermal findings included thickening of the skin due to hyperplasia and hyperkeratosis of the epidermal epithelium, and dilation and plugging of hair follicles with keratinous material. Microscopic study of liver sections showed a considerable diversity of lesions, including centrilobular degeneration, focal hydropic degeneration, focal necrosis, atrophy of centrilobular parenchymal cells, cytoplasmic hyaline degeneration, pigmentation of Kupffer cells, and, to a lesser extent, pigmentation of parenchymal cells. Renal damage was found in all PCB-treated animals. The most common findings were hydropic degeneration of the convoluted tubules, with nuclear pyknosis, and bursting and lysis of the tubular epithelial cells [208]. Dilation of the renal tubules, filled with casts of necrotic epithelial cells, was found in half the rabbits. These findings indicate that the dermal application of PCB mixtures causes systemic lesions of the liver and kidneys besides the direct effect on the skin. However, in other studies [19,21,23], it was determined that Clophen A60, Phenoclor DP6, and Aroclors 1248 and 1254 were contaminated, to varying degrees [21], with highly toxic tetra- and pentachlorodibenzofurans.

(b) General Effects of Oral Administration of PCB Mixtures

Single-dose oral LD50's of several PCBs reported in rats (JW Cook, written communication, June 1970) generally indicated that as the degree of chlorination increased, the acute toxicity decreased. The more highly chlorinated products (Aroclors 1248-1268) were of approximately equal toxicity (LD50's of about 10 g/kg), whereas the LD50's of the less highly chlorinated products (Aroclors 1221-1242) ranged from about 4 to 9 g/kg.

The minimum lethal doses of these PCBs applied to the skin of rabbits were generally in the range of 1-2 g except for 1221 which was in the range of 2-3 g and 1268 which was >2.5 g.

The immunosuppressive activity of Aroclor 1260 was described in 1972 by Vos and de Roij [209] who fed three groups of 12 4-week-old female albino guinea pigs the PCB at 0, 10, or 50 ppm in their diets for 8 weeks. Aroclor 1260 was analyzed and found to be free of contamination with chlorinated dibenzofurans, although it appeared to contain a minor acnegenic impurity. In each group, six animals received sc injections of aluminum phosphate-adsorbed tetanus toxoid to stimulate the lymphoid system (antitoxin production). The other six animals in each group served as positive or negative controls. Cellulose acetate electrophoresis was used to determine serum proteins, including gamma-globulins. The gamma-globulin-containing cells in the popliteal lymph nodes were significantly reduced in the stimulated animals fed PCBs. Serum gamma-globulin levels were significantly decreased in the guinea pigs stimulated with tetanus toxoid and fed 10 ppm of Aroclor 1260 in their diet. Increased serum alpha-globulin levels were found in the stimulated guinea pigs fed either 10 or 50 ppm of the PCB, and significantly increased concentrations of albumin were found in the sera of both the stimulated and the unstimulated guinea pigs fed the PCB at 10 ppm. Also, both the absolute and relative weights of the cervical lymph nodes in the unstimulated group fed 10 ppm of Aroclor 1260 were significantly reduced whereas those of the mesenteric lymph nodes in the stimulated guinea pigs fed 10 and 50 ppm of PCB were significantly increased. These findings indicate that some immunosuppressive effect was produced by the feeding of PCBs. However, the

decreases of the gamma-globulin levels were not found to be dose-related. No evidence of any PCB-induced change was observed in microscopically examined stained sections of liver, kidneys, adrenals, and skin.

Vos and Van Driel-Grootenhuis [210] reported their studies of the effects of PCBs on the humoral and cell-mediated immunities of guinea pigs in 1972. Three experiments were performed with different protocols. In the first experiment, the authors investigated the humoral immune response of guinea pigs fed 0, 10, 50, or 250 ppm of Clophen A60 in their diets and stimulated with a single sc injection of tetanus toxoid 3 weeks after the feeding regimen began. Suppression of humoral immunity was observed at the 50-ppm level. Microscopic examination of the livers revealed centrilobular degeneration, cellular atrophy, cellular necrosis, and nuclear enlargement. At the 250-ppm level, no antitoxin production was observed.

The diets of guinea pigs in a second experiment [210] contained 0, 10, or 50 ppm of Clophen A60 or 50 ppm of Aroclor 1260. Primary and secondary antigenic stimulations with tetanus toxoid were given after 3 and 5 weeks. The experiment lasted 6 weeks. Suppression of the humoral immune response was observed in groups fed 50 ppm of either PCB product. Decreases in the weights of the thymuses and increases in liver weights were observed at the 50-ppm Clophen A60 level and, to a lesser degree, at the 50-ppm Aroclor 1260 level. The residual liver concentrations of PCBs increased as the dose level and the duration of exposure increased.

In their third experiment, Vos and Van Driel-Grootenhuis [210] fed 30 guinea pigs diets containing Clophen A60 at 0, 50, or 250 ppm. After 3 weeks, the animals were challenged with 0.05 ml of Freund's complete adjuvant followed in 47 days by an injection of 0.1 ml of avian tuberculin

antigen. The animals were killed 2 days later. All animals of the 250-ppm group died during the experiment, exhibiting retarded growth, atrophy and depletion of the lymphoid system, and liver damage. Significantly decreased thymus weights and increased liver weights also were observed, and total white blood cell counts were reduced significantly.

Bruckner et al [211] administered Aroclor 1242 to rats by oral intubation to determine its acute and subacute effects. Two groups of six rats each were given single doses of either 2.5 or 6.0 g/kg of Aroclor 1242. The initial effects observed at both dosages were the same for the first 4 hours, ie, diarrhea, decreased spontaneous activity and muscle tone, decreased response to pain stimuli and mild chromodacryorrhea. However, during the next 24 hours the group receiving 6.0 g/kg had profuse diarrhea, adipsia, oliguria, anorexia, erythema of the limbs, lack of response to pain stimuli, and general weakness. Eventually, ataxia, coma, and death followed. The condition of the rats receiving the lower dosage gradually improved after the first 24 hours and was normal at the end of 72 hours.

Another group of rats [211] that received single oral doses of 4 g Aroclor 1242/kg showed weight loss, elevated packed red cell volumes, increased serum polymorphonuclear leukocytes, crenated red blood cells, and increased SGOT activities. All organs appeared normal except the livers and kidneys. The livers exhibited foci of sudanophilic vacuolation. The kidneys showed widely scattered foci of vacuolated tubular epithelial cells.

In the subacute studies conducted by Bruckner et al [211], six rats were given 100 mg of Aroclor 1242/kg orally every other day for 3 weeks;

three control rats received 100 mg/kg of peanut oil on the same schedule. Changes observed in the treated rats included increased liver weights, decreased packed red blood cell volumes, increased SGOT activities and marked increases in liver microsomal hydroxylating and N-demethylating enzyme activities. Microscopic examinations of the livers and kidneys revealed greater increases in generalized lipid vacuoles than had been found with the acute doses. When single ip doses of 100 mg Aroclor 1242/kg were given to rats, increased N-demethylase and aniline hydroxylase activities were observed after only 24 hours [211].

The effects of lower dietary levels of Aroclor 1242 on rats were reported by Bruckner et al [212], who fed it to groups of six male Sprague-Dawley rats at concentrations of 0, 5, or 25 ppm for 2, 4, or 6 months. Small reductions in hematocrit and hemoglobin levels similar to those found in other studies by Bruckner et al [211,213] were seen. Urinary excretion of coproporphyrin was significantly increased and dose-dependent increases in liver microsomal hydroxylase activity were measured at the time of each sampling. Similar dose-dependent relationships were found in other experiments [213,214]. Proliferation of the hepatic endoplasmic reticulum was seen after 2 months of ingesting a diet containing 25 ppm of Aroclor 1242; after 4-6 months of such ingestion, lipid vacuolization of the liver was evident.

In 1974, Bruckner et al [213] reported reduced weight gain, hepatic and renal damage, and an increase in urinary coproporphyrin excretion in rats injected ip with a total of 1.6 g/kg of Aroclor 1242 during a 10-week period. The hydroxylating and N-demethylating activities of the liver were significantly elevated 24 hours after a single ip injection of 100 mg/kg of

Aroclor 1242, with hydroxylating activity showing the greater increase. Cytochromes P-450 and b5 and NADPH-cytochrome reductase activities of the liver all were significantly increased 3 days after dosing. A single dose of 50 mg/kg gave similar but less marked effects, whereas 25 mg/kg produced significant increases in only hydroxylating and N-demethylating activities.

Kimbrough et al [215] described in 1972 some effects of chlorobiphenyl mixtures on the livers of rats. Groups of 3- to 4-week-old male and female Sherman strain rats were given Aroclors 1254 and 1260 at 0, 20, 100, 500, and 1,000 ppm in their diets. Each group consisted of 10 males and 10 females. Food consumption was measured periodically. The animals were fed the PCBs for 8 months.

One female fed 100 ppm of Aroclor 1260 died after 6 months and two females fed 500 ppm died after 1 and 2 months. Eight females fed the 1,000 ppm diet died in 2-6 months. The rats fed 500 and 1,000 ppm gained less weight than did the controls. At autopsy, livers of the male rats fed Aroclor 1260 weighed significantly more than did livers of control male rats. Livers of the females were larger than normal but the weight differences were not statistically significant, although at 500 ppm the fractions of body weight represented by the livers increased significantly as a result of the reduced body weight gain. Microscopic findings in the livers included hepatocytic hypertrophy, inclusions in the cytoplasm, brown pigment in the Kupffer cells, lipid accumulations, and, at the higher dietary levels, adenofibrosis. The nodular greyish-white areas that represented extensive foci of adenofibrosis consisted of fibroblasts and collagen that surrounded rosettes of epithelial cells [215].

Aroclor 1254 produced similar results [215]. One female and two male rats died at 500 ppm, but none died at the lower dietary levels, and the rats fed 500 ppm gained less weight than did controls. Ultrastructural changes in the livers of exposed animals consisted of proliferation of the smooth endoplasmic reticulum (SER) and atypical mitrochondria. Similar changes have been observed in other experiments with PCBs [131,212,216-218]. A major difference between the effects of the two products was the much higher incidence of hepatic adenofibrosis at a lower dietary level of Aroclor 1254 (100 ppm). In general, the effects of Aroclor 1254 on the liver were more pronounced than those of Aroclor 1260.

In 1973, Kimbrough et al [219] reported the results of a study conducted to determine whether morphologic changes produced in the liver would regress after ingestion of PCBs was stopped. Fifty male SPF Sherman strain rats were given Aroclor 1254 at 500 ppm in their diets for 6 months, and then were returned to normal diets. Groups of five rats were killed 0, 1, 2, 3, 4, 6, 8, and 10 months after ingestion of PCBs had been discontinued. The livers and adipose tissues of the 10-month group were analyzed for PCBs. Control animals were used in all evaluations and were fed only laboratory chow.

The livers of exposed animals killed when exposure to Aroclor 1254 was discontinued were enlarged and most of the 40 livers studied microscopically showed enlarged hepatocytes, increased lipid contents, and adenofibrosis. A brown pigment was seen in the Kupffer cells and in other macrophages of 15 livers. Small adenofibrotic lesions consisting of glandular epithelial cells that formed ducts surrounded by slight amounts of fibrotic tissue were seen; larger lesions had more extensive fibrosis

and contained collagen. Similar findings had been reported earlier by Kimbrough et al [215] and both in the areas of adenofibrosis and in othewise normal hepatic tissue, Kimbrough [220] later reported finding small clusters of glandular type cells, resembling those of pancreatic tissue in general appearance and staining characteristics, in 15 of 36 rat livers with adenofibrosis. The cells stained red with the stain used for esterase, and developed a blue granular appearance with the stain used for protein bound tryptophan. These staining reactions were suggestive of those of salivary gland tissue to the author. The cells may have been derived from either ductal or vascular epithelium, but there is no evidence to substantiate either suggestion [220].

Signs of regression of the adenofibrotic lesions were not noted except for the disappearances of epithelial cells from the centers of the lesions [219]. Liver weights did regress to normal after 10 months without PCB exposure, but the lipid accumulation and the hypertrophy of the hepatic cells remained unchanged. Analysis of adipose tissues from the final group of rats killed showed that the PCB levels ranged from 924 to 1688 ppm (mean, 1192 ppm). Liver PCB levels ranged from 17.3 ppm to 26.2 ppm (mean, 22.7 ppm). The PCB concentrations in the adipose tissue and livers of the control animals were less than 1.0 ppm. The authors [219] were unable to decide whether contamination of Aroclor 1254 with dibenzofuran was responsible for the observed liver lesions [219].

Kimbrough and Linder [221] reported in 1974 the results of a PCB-feeding experiment which they undertook to induce adenofibrotic lesions in the livers of BALB/cJ inbred male mice, ie, lesions similar to those previously observed in rats fed Aroclors 1254 and 1260. Two hundred mice,

5-6 weeks old, were distributed randomly into 4 groups of 50 each. Two groups were fed a ground diet containing 300 ppm of Aroclor 1254. At the end of 6 months, one of these exposed groups was placed on a PCB-free diet while the other exposed group continued on the test diet for another 5 months. The other two groups served as controls and were fed the plain ground laboratory diet for the entire 11 months. The average PCB intake was 49.8 mg/kg/day in the mice fed the PCB diet for the entire 11-month period.

Livers of 45 of the 58 surviving control mice were found to be normal. The other 13 had focal round-cell infiltrates and sometimes small areas of necrosis and fibrosis. Skin abscesses, usually near the groin, were identified in all 13 control mice with hepatic round-cell infiltrates. These skin lesions were thought to be abscesses of the preputial glands. In comparison, 10 hepatomas were identified in 9 of the 22 survivors in the group fed the PCB diet for 11 months. These tumors consisted of well-differentiated hepatocytes, relatively uniform in size but usually smaller than the surrounding liver cells. They were well circumscribed and surrounded by compressed hepatic parenchymal cells or strands of fibrous tissue. In the larger tumors, there were areas in which the sinuses were dilated and filled with a pink-staining amorphous material. No metastases were seen on gross inspection of the organs, although no detailed screening, such as serial sectioning of the lungs, was undertaken. Only one small hepatoma, composed of well-differentiated hepatocytes, was identified among the 24 survivors fed the PCB diet for only 6 months. The authors [221] noted that the mouse strain used in this study rarely develops hepatomas spontaneously.

Adenofibrosis was identified in the livers of the mice subjected to the 11-month PCB diet. These lesions, according to the authors [221], may or may not be precursors of malignant lesions. They were seen in several areas of each liver as fibrosis and as glandular formations of proliferated epithelial cells replacing parenchymal cells. They formed ducts which produced mucus and which were surrounded by connective tissue of varying abundance.

Allen and Abrahamson [216] reported on morphologic and biochemical changes in the livers of rats given Aroclors 1248, 1254, and 1260 at 1,000 ppm in their diets for 6 weeks. A control group was fed unsupplemented chow. Each group contained 24 rats. Four rats were killed after 1,3,7,14,21, 28, and 42 days of exposure. White blood cells, hemoglobin (Hb), hematocrit (Hct), differential white cell count, total serum proteins, and blood urea nitrogen (BUN) were measured at each interval. Selected tissues were prepared for microscopic examination and the livers were homogenized and analyzed for cytoplasmic protein, DNA, and RNA. Liver microsomes were examined for aryl hydrocarbon hydroxylase, nitroreductase, N-demethylase, and nitrophenylacetate hydroxylase activities, and for cholesterol content.

All of the PCB-dosed rats failed to gain weight. The most severe effects were noted with Aroclor 1248, followed by Aroclors 1254 and 1262. All blood samples showed increases in Hb and Hct, with no changes in the white blood cell counts. Neutrophils were increased in the sera of all treatment groups. There were no changes in BUN or in total serum protein levels. Organ weights in all of the experimental rats constituted higher percentages of total body weights than in controls; the livers showed four-

fold increases in weight in all PCB-dosed groups. The increased liver weights were discernible after only 1 day of exposure. Liver hypertrophy was attributed to proliferation of SER, development of large concentric arrays of membranes, and increases in lipid droplets within the cytoplasms of the affected cells. Aroclor 1248 was the most, and 1262 the least, toxic PCB product as judged by changes in liver histology. Liver homogenates contained increased concentrations of protein and RNA, and decreased concentrations of DNA. These changes were seen within 2 weeks after initiation of feeding Aroclors 1248 and 1254; the protein and nucleic acid concentrations then either leveled off or decreased after 4-6 weeks. Aroclor 1262, however, did not induce these effects until 4 weeks after the beginning of feeding; the initial changes were followed by reversals [216].

Liver microsomal fractions had increased protein and phospholipid concentrations. These changes occurred earlier in the rats fed Aroclor 1248 and 1254 than in the Aroclor 1262-fed animals. Microsomal nitroreductase activity, expressed on the basis of microsomal protein, was variable in the animals fed Aroclors 1248 and 1254; however, all animals fed Aroclor 1262 had progressive increases in nitroreductase activity. N-demethylase activity increased early in the experimental period and increased progressively in all treatment groups as the exposures continued. Aryl hydrocarbon hydroxylase, glucose-6-phosphatase, and other esterase activities increased initially, followed by continuous declines in activity in the groups fed Aroclors 1248 and 1254. The rats fed Aroclor 1262 had similar, but less marked, changes. When enzyme activities were expressed as activity per total liver, all showed marked increases. After 6 weeks, other degenerative changes in the liver occurred, such as dissolution of

the concentric arrays of membranes, vesiculation of the endoplasmic reticulum, and accumulation within the cytoplasm of lipid droplets that had developed during the hypertrophic phase of the intoxication [216].

Allen et al [222] administered PCBs (Aroclor 1248) and polychlorinated terphenyls (Aroclor 5460) to rhesus monkeys. Sixteen male monkeys were distributed into three groups. Group 1 consisted of six animals and was fed a basal diet supplemented with 300 ppm of Aroclor 1248. Group 2 consisted of six animals fed a basal diet containing 5,000 ppm of Aroclor 5460. The remaining four monkeys constituted the control group and were fed the standard colony diet. Each animal had access to 400 g of diet daily. The physical status of the animals was evaluated daily and "complete" blood studies were performed biweekly. Approximately 10 g of liver tissue were removed from each animal during the 6th week (via laparotomy) and during the 12th week (at necropsy) for biochemical and microscopic evaluation.

Animals fed Aroclor 1248 lost an average of 26% of body weight; those receiving Aroclor 5460 lost an average of 19% of body weight. Gross changes seen in the animals fed Aroclor 1248 included loss of hair from head, neck, and back, puffy faces, edematous lips, and swollen eyelids with purulent exudates around the eyes. These changes were seen within 1 month; similar, but less marked changes were seen in the animals fed Aroclor 5460 [222].

Hematologic changes developed gradually. After 3 months, hemoglobins had decreased by about 2 g/100 ml, and hematocrits had diminished from about 40% to 33%. Total white blood cell counts did not change; however,

there was a shift to the left in the differential count. Total serum proteins decreased by about 1.5-2 g/100 ml, and there was a gradual shift in the albumin/globulin ratio of the serum protein. No changes were seen in SGOT or BUN. Both experimental groups had similar patterns of changes [222].

At necropsy, both groups of animals showed extensive alopecia, acneiform lesions of the skin, subcutaneous edema, liver hypertrophy with fatty infiltration, and gastric mucosal hypertrophy and hyperplasia. Liver hypertrophy was attributed mainly to proliferation of the hepatocytic SER. Gastric hypertrophy was characterized by thickened gastric mucosa, numerous large cystic areas filled with mucin, and hyperplasia. Ulceration was seen, as was invasion of the underlying mucosa by glandular-type epithelial cells [222].

The authors [222] noted that, biochemically, the decreases observed in the DNA concentrations of the livers of both experimental groups reflected the hypertrophic cells observed microscopically, and the increases in protein and RNA were said to be compatible with the proliferated SER. Levels of microsomal protein per gram of protein were not markedly altered, but decreases in the specific activities of esterase, aniline hydroxylase, nitroreductase, and glucose-6-phosphatase became apparent within 6 weeks, and persisted. N-demethylase activity was increased at each examination.

The results of a toxicity study conducted on four young male rhesus monkeys fed Aroclor 1242 at concentrations of 3, 10, 30, or 100 ppm were reported by Bell [223], McNulty [94], and in a communication written in

March 1977 by WP McNulty. All four monkeys on these diets died within 9 months. The monkeys had facial swelling, red and swollen eyelids, conversion of all secretory-type cells of the stomach to mucous cells, growth of mucous glands into the muscular walls of the stomach, multiple ulcers in the stomach, atrophy of the thymus gland, and either disappearance of sebaceous glands or conversion of these glands to keratin cysts, particularly in the eyelids.

Allen et al [104] and Allen and Norback [224] found gastric hypertrophy and hyperplasia and focal ulceration of the stomach lining in adult male rhesus monkeys fed single doses of 1.5 or 3.0 g of Aroclor 1248.

(c) Studies Involving Individual PCB Isomers

In 1972, Vos and Notenboom-Ram [225] discussed the comparative toxicities of dermally applied 120-mg doses of 2,2',4,4',5,5'-hexachlorobiphenyl and Aroclor 1260. The PCBs, dissolved in isopropanol, were applied 20 times to the clipped and shaved backs of three groups of four rabbits each, 5 times/week for 4 weeks. The control group received only isopropanol. The Aroclor 1260 sample was found to be "free" of chlorinated dibenzofurans (limit of detection, 1 ppm), and because of the nature of the chemical reactions utilized in the synthesis of 2,2',4,4',5,5'-hexachlorobiphenyl. Dermal applications of these PCBs resulted in early macroscopic skin lesions and morbid liver changes similar to those found by Vos and Beems [208]. These liver lesions included centrilobular degeneration and liver cell atrophy, focal cytoplasmic hyaline degeneration of the hepatocytes, enlarged nuclei, loss of glycogen and proliferation of SER. The authors [225] concluded that, although the

major acnegenic action of crude PCB mixtures results from the presence of chlorinated dibenzofurans, PCBs have acnegenic actions of their own.

Hansell and Ecobichon [217] described the effects of a series of chemically pure chlorobiphenyls on rat liver morphology. Biphenyl and a series of isomerically pure mono-, di-, tetra-, hexa-, and octachlorobiphenyls of known composition were administered intraperitoneal (ip) injections in daily doses of 50 mg/kg to groups of seven young male Woodlyn strain Wistar rats for 3 consecutive days. The rats were killed 4 days after the final injections had been made and sleeping times after phenobarbital administration had been determined. The major microscopic alterations observed were proliferation of the hepatocytic SER, changes in the rough endoplasmic reticulum, and increased numbers of lipid droplets and microbodies. However, in the rats administered isomerically pure hexa- and octachlorobiphenyls, there were additional changes in hepatic morphology, including large numbers of hepatocytes with cytoplasmic vacuoles and small foci of necrosis involving five or six cells. Biphenyl and 2,2'-dichlorobiphenyl appeared not to induce extensive proliferation of the SER, but with the other compounds the SER proliferation appeared to be related to the degree of chlorination, especially to the presence of a chlorine in the 4 and/or 4' positions.

The investigators [217] also injected groups of seven rats ip with daily doses of 100 mg/kg of either 4-mono-, 4,4'-di-, or 2,5,2',5'-tetrachlorobiphenyl for 7 days; all rats from each group were killed 24 hours after the last injections. This regimen caused more pronounced alterations in hepatocytic ultrastructure than those observed after the 3-day experiments. Marked proliferation of SER and increased numbers of

microbodies and lipid droplets were noted, as were a large number of necrotic foci, centrilobular necrosis, and proliferation of biliary ductules. The most severe lesions were seen in the group receiving 4,4'-dichlorobiphenyl. There was not the increase in hepatic weight and cell size noted by other authors [219,226]. Hansell and Ecobichon [217] observed that this apparent anomaly may have been due to the duration of PCB administration, only 3-7 days, and to the relatively low dosages.

Allen et al [226], in a series of experiments, studied the toxicity of 2,2',5,5'-tetrachlorobiphenyl in rats and rhesus monkeys. In the first experiment, 5-week-old rats were separated into five groups consisting of five males and five females each. After being fasted overnight, each of these groups was administered by gavage a single dose of one of the following amounts of the compound dissolved in corn oil: 0, 0.5, 1.0, 1.5, or 2.0 g/kg. Each dose had a total volume of 1 ml. After the dose had been given, blood was taken for complete blood counts. Eighteen of the 40 PCB-dosed rats died within 3 days. The dead animals included all those given 2.0 g/kg, 7 rats from the group given 1.5 g/kg, and 1 rat from the 1.0 g/kg group. The rats were observed for 21 days after which complete blood counts were performed and the surviving animals were killed and necropsied. The major pathologic changes observed in the animals that died were marked regressions of lymphocytes in the spleen and lymph nodes and of cortical thymocytes. With the exception of hemorrhage and atrophy of the thymus, which were related to the decrease in the cortical thymocytes, and enlargement of the liver and kidneys, all tissue samples were normal when compared with those from the nonexposed group.

The second experiment was conducted to determine the effects of hepatic microsomal enzyme activity on the responses of male Sprague-Dawley rats to 2,2',5,5'-tetrachlorobiphenyl [226]. The rats were separated into 6 groups of 10 animals each. Group 1 was a positive control given four ip injections of 75 mg/kg of the microsomal enzyme inhibitor, SKF 525A, at 8-hour intervals. Group 2 was given four daily sc injections of 75 mg/kg of phenobarbital. Group 3 was the general control. Groups 4, 5, and 6 received 1.25 g/kg of the chlorobiphenyl in 1 ml of corn oil by gastric intubation; group 5 was dosed with the chlorobiphenyl 24 hours after 4 days with daily sc doses of 75 mg/kg of phenobarbital. Group 6 received 75 mg/kg of SKF 525A ip 2 hours prior to administration of 2,2',5,5'-tetrachlorobiphenyl, and every 8 hours thereafter for 24 hours. Within 4 days, all the rats in group 5 and 50% in group 4 had died whereas none had died in group 5, which was pretreated with phenobarbital. The findings that pretreatment with phenobarbital allowed complete survival of rats after an LD50 dose of the PCB, whereas pretreatment with SKF 525A caused 100% mortality, indicated to the investigators that metabolic transformation by endoplasmic reticulum enzymes resulted in deterification [226].

The third experiment was performed to compare the differences between the responses of rats to 2,2',5,5'-tetrachlorobiphenyl and Aroclor 1248 [226]. Twelve male rats were placed in three groups of four animals each; group 1 was fed 100 ppm of Aroclor 1248 in its diet, group 2 was given 100 ppm of the 2,2',5,5' isomer in its diet, and group 3 (control) was fed the staple ground meal diet. After 4 weeks of these feeding regimens, the rats were fasted for 24 hours, killed, and necropsied; blood was also collected

at this time. There were no detectable differences in general appearance, food consumption, activity, growth rate, or blood chemistries between the two experimental groups and the control group. The only significant difference at autopsy was an increase in liver weight as a percentage of body weight of the animals fed Aroclor 1248: 4.46 ± 0.11%, compared with 3.38 ± 0.13% for those fed 2,2',5,5'-tetrachlorobiphenyl and 3.0 ± 0.12% for the controls. Proliferation of the endoplasmic reticulum within the hepatic cells was observed in the Aroclor 1248-fed animals. The N-demethylase activity and the cytochrome P-450 content of the microsomal fraction of homogenized liver from the Aroclor 1248-fed rats were increased whereas the activity of aniline hydroxylase and glucose-6-phosphatase in this fraction had decreased. There were increases in the concentrations of protein and RNA and a decrease in the DNA content of the liver homogenates from the Aroclor-fed animals. The livers of rats fed the isomer had similar but less marked changes in concentrations of protein, RNA, and DNA. The mean concentration of microsomal protein in liver was identical with that of the controls but was more variable. A significant increase in the mean activity of N-demethylase was found.

The fourth experiment [226] was performed to determine the effects of 2,2',5,5'-tetrachlorobiphenyl on non-human primates. Seven adult male rhesus monkeys were given 18 mg/kg of the PCB isomer dissolved in 2.5 ml of corn oil and three controls were given 2.5 ml of corn oil, all by gavage after a 24-hour fast. Complete blood counts had been done on each animal prior to intubation. Immediately after treatment, the animals were placed in metabolism cages and their urine and feces analyzed for 2,2',5,5'-

tetrachlorobiphenyl by GLC. On the 14th day of the experiment the animals were fasted for 24 hours, killed, and necropsied. Tissues were taken for light and electron microscopy and GLC. No overt clinical effects were seen in the treated monkeys. Over 12% of the PCB was recovered unmodified in the feces but only minute amounts were present in the urine. Only the adipose tissue and adrenals had high PCB assays, and microscopically, except for a moderate proliferation of hepatocytic SER, all tissues were normal. DNA was slightly decreased in liver homogenates of exposed animals and hepatic microsomal cytochrome P-450 was increased.

Torok [227], in 1976, reported a study of pregnancy in NMRI mice treated with 2,2'-dichlorobiphenyl. The mice were separated into three groups, with group 1 (37 mice) serving as the control; group 2 (18 mice) received the PCB in oral doses of 375 mg/kg/day on days 1-3 of gestation, and group 3 (37 mice) received 750 mg/kg/day on the same schedule. Administration of the PCB at either level resulted in longer intervals from breeding to parturition: 18.2 days for controls, 19.4 days for mice treated with the PCB at 375 mg/kg/day, and 21.8 days in mice treated at 750 mg/kg/day. There also were reductions in the numbers of dams with litters and in the mean litter size. Reductions were greatest in group 3. The authors [227] concluded that the effects were due to delayed implantation.

(d) Reproductive and Teratogenic Effects of PCB Mixtures

Curley et al [101] studied the transplacental movement of PCBs in rats. Three groups of 90-day-old female rats were paired with male rats of the same age. All were fed standard laboratory chow by the authors, who designated the day of insemination as day 0. Aroclor 1254 was given in

doses of 0, 10, and 50 mg/kg in peanut oil (presumably by oral intubation) once a day on days 7 through 15 of pregnancy to groups 1, 2, and 3, respectively.

On the 20th day after insemination, fetuses were taken by Caesarean section from three rats of each PCB-exposed group and from two control rats. The fetuses from each experimental group were divided into two lots and analyzed as duplicate samples for PCBs. Ten rats from each of the three experimental groups were allowed to deliver spontaneously. When the offspring were 5 days old, six mothers from each exposed group and two from the control group were separated from their litters for several hours and then returned for 1 hour of nursing. The contents (milk) of the pups' stomachs were analyzed for PCBs. Litters from three rats in each experimental group were allowed to survive until weaning at 21 days of age. These baby rats then were killed and their tissues analyzed for PCBs by GLC with EC detection [101].

The rats subjected to Caesarean section had normal fetal complements, in both size and number, and in the rats allowed to deliver spontaneously there were no significant differences in average litter size and weight among the three groups. However, the three groups, with 0, 10, or 50-mg/kg doses of Aroclor 1254, had 0, 1, and 4 stillbirths on the average, respectively [101].

Analyses of the tissues of the Caesarean-delivered pups showed measurable levels of PCB-derived components in the exposed groups only. The mean PCB concentrations were <0.12, 0.63 ± 0.06, and 1.38 ± 0.06 $\mu g/g$, respectively, for groups 1, 2, and 3. Although the PCB dose for group 3 was five times that of group 2 (50 vs 10 mg/kg Aroclor 1254), the average

amount of PCB-derived components measured in the fetuses from these two groups differed by only two-fold. The authors suggested that the PCB distribution between the mothers and their fetuses may not have been uniform and that a partial (unspecified) barrier may have existed [101].

Livers were increased in size and had enlarged hepatocytes in 15 of 21, and 15 of 20 weanling rats from groups 2 and 3, respectively. Some rats from group 3 showed vacuolization of the hepatocytic cytoplasm (10 of 20), and 5 of 20 exhibited bile duct proliferation. Morphologic changes in the liver were more pronounced at the higher dose [101].

PCB-derived components were detected in litter-pooled milk at mean concentrations of <0.75, 20.60 ± 1.59, and 66.34 ± 8.36 μg/g in groups 1, 2, and 3, respectively. The authors [101] concluded that the morphologic changes identified in the livers of exposed weanling rats were probably caused by PCBs transmitted to the pups in the milk from the dams [101].

Reproductive effects of feeding Aroclors 1254 and 1260 to Sherman strain rats for two generations were reported by Linder et al [228] in 1974. In preliminary experiments, the two mixtures were fed to groups of 10 females at 100 and 500 ppm in the diet. Two similar groups served as controls. The diets, fed for 67 days before the first mating, were continued through gestation and lactation. After weaning of the first litters, the parents continued to be fed the diets. After being fed PCBs for a total of 186 days, they were again mated and the diets fed through the second gestation and lactation. Feeding Aroclor 1254 at 500 ppm resulted in only 4 first litters being born (2 alive), with no survivors to weaning. Consequently, feeding Aroclor 1254 at 500 ppm was discontinued. With Aroclor 1254 at 100 ppm, there was little indication from either the

first or second mating of reduced fertility, or increased death in utero or during the nursing period. Aroclor 1260 fed at 100 ppm for 67 days had no apparent effect on reproduction, litter size, or survival of the first litters; however after being fed for 186 days, only 5 second litters were born from 9 mated females, compared to 9 out of 10 in the controls. At 500 ppm, Aroclor 1260 resulted in more pups dead at birth in first litters, reduced second litter size, and fewer litters being weaned (3 of 8 first litters; 2 of 6 second litters).

Subsequently, Linder et al [228] fed groups of 20 female Sherman rats Aroclor 1254 at 1, 5, 20, and 100 ppm and Aroclor 1260 at 5, 20, and 100 ppm. Appropriate control groups with similar numbers of rats were used. The experimental plan was similar to that described above except that the F1 generation pups continued to be fed the F2 generation diet until they reproduced the F2 generation. The first matings of the F1 generation were after they were exposed to PCBs in utero, in milk, and in the diet for 125-129 days. The F1 generation rats fed Aroclor 1254 at 20 and 100 ppm were mated a second time after a total of 274 days of PCB exposure.

Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups in their litters than the controls [228]. There were also fewer F2 generation pups than controls. Second litters born from rats fed 100 ppm of Aroclor 1254 had increased mortality and markedly decreased mating performance. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 21-day-old F1 male weanlings at 1 ppm of Aroclor 1254 and in either sex of F1 and F2 weanlings at 5 ppm or higher of both Aroclors 1254 and 1260.

Villeneuve et al [229], in 1971, wrote about fetotoxic effects of Aroclors 1221 and 1254 in rabbits. Twenty-four mature female rabbits were divided into six groups each comprised of two control and four experimental does. Experimental animals were given the Aroclors orally at 1.0 and 10 mg/kg/day beginning after mating and continuing through gestation.z They were killed 28 days after mating. Neither Aroclor had a fetotoxic effect at either dose level. Liver weights expressed as percentages of body weight were significantly heavier in the does fed 10 mg/kg Aroclor 1254 than in controls (4.29 vs 2.79%). Liver weights of the other experimental does and of all fetuses were not significantly different from controls.

In another experiment reported by Villeneuve et al [230], 16 mature female rabbits were separated into four groups and, after mating, were dosed orally with 0, 12.5, 25, or 50 mg/kg of Aroclor 1254 daily for the first 28 days of gestation. The 50-mg/kg group had three pregnancies.z One pregnant female died on the 11th day and contained nine fetuses. A second one died on the 17th day and contained six resorption sites. The third female aborted three dead fetuses on the 28th day. Two rabbits in the 25-mg/kg group had normal fetuses. A third rabbit aborted her litter on the 28th day and died. The four animals in this group showed an average weight loss of 26 g and liver weights found at autopsy 29 days after mating averaged 5.11% of their body weights. Autopsies were performed on the fetuses of the two rabbits which had conceived in the 12.5-mg/kg group; the first rabbit had two normal fetuses, with six resorption sites, while the second aborted two fetuses on the 27th day and was found on autopsy to contain one partially resorbed and two dead fetuses. The two dead fetuses obtained on autopsy showed subcutaneous cephalic hemorrhages and had

asymmetric skulls. Average weight gain of the females during pregnancy was 265 g, and their liver weights averaged 6.03% of their body weights. Controls gained 654 g during pregnancy, and their livers averaged 2.66% of their body weights.

In their third experiment, Villeneuve et al [230] gave each animal in three groups of six female rabbits 25 mg/kg Aroclor 1254 in corn oil (orally). Controls received corn oil alone. A control group and one exposure group were dosed from day 1 through day 28, and another control and an exposure group were dosed from day 7 through day 28. Administration of Aroclor 1254 at 25 mg/kg/day from day 1 through day 28 resulted in abortions in two of four rabbits. Their average weight gain was 245 g and liver weights averaged 6.03% of their body weights (controls, 531 g weight gain; liver weights, 2.65% of body weight). In the second group administered PCBs, one of four rabbits aborted. Average weight gain was 156 g and the liver weights averaged 4.82% of the body weights.

PCB concentrations in various tissues of six rabbits and their fetuses, chosen at random from the above groups, were determined by Grant et al [231]. Concentrations of PCBs were higher in the livers of the fetuses than in their mothers: 5.4 vs 2.1 ppm at a dose of 1 mg/kg; 78.1 vs 69.5 ppm at 12.5 mg/kg; and 375.3 vs 124.2 at 25 mg/kg. In other tissues, PCB concentrations were generally less in the fetuses than in the dams.

Allen et al [232] in 1974 presented evidence of possible fetotoxicity in a short term PCB feeding experiment with 12 adult female rhesus monkeys. The monkeys ranged in age from 7 to 10 years and had an average weight of 5.6 kg. Each previously had delivered at least one infant and the menstrual cycle of each had been recorded for at least 2 years. Six of the

monkeys were fed an unspecified diet containing 25 ppm of Aroclor 1248 for 2 months. The other six monkeys were controls. After 2 months, the PCB-exposed animals were placed on PCB-free diets of commercial monkey chow.

The monkey which had consumed the largest amount of PCBs died on the 128th day of the experiment [232]. At the beginning of the 5th month, ie, 3 months after the test animals had been removed from the PCB-containing diet, the remaining five test monkeys and the six controls were mated again. Three PCB-exposed monkeys were thought to have conceived since each showed the characteristic post-conceptual bleeding, lack of the subsequent menstrual period, and an enlarged uterus. Two either aborted or reabsorbed their fetuses during the 2nd month of pregnancy and the third monkey delivered a well-developed but smaller-than-normal (375 g ± 1 g vs 544 g ± 101 g) infant. High concentrations of PCBs were found in the adipose tissues and adrenals of this infant with means of 27.7 and 24.4 μg/g, respectively, as compared to a range from 0.01 to 0.98 μg/g in the other infant tissues analyzed. The PCB levels in the placenta averaged 0.9 μg/g, and 50 μg/g in the mother's adipose tissue. Two additional attempts were made to breed the nonpregnant females during the next 5 months. Only the controls became pregnant and all delivered normal infants. Six of the monkeys were fed an unspecified diet containing 25 ppm of Aroclor 1248 for 2 months. The other six monkeys were used as controls. After 2 months, the PCB-exposed animals were placed on PCB-free diets of commercial monkey chow.

In 1976, Barsotti et al [233] and Allen and Barsotti [234] reported their findings on reproductive dysfunction in female and male adult rhesus monkeys fed Aroclor 1248 in their diets. One test group of female monkeys

received 2.5 ppm and the other group received 5.0 ppm of Aroclor 1248 in a commercial diet. The control group, containing 12 females and 6 males, was fed the commercial diet alone. Females received 200 g, and the males received 300 g of their respective diets daily. Food left in the feeding cups at the end of the day was removed and weighed to determine the daily intake. After the end of the first 6 months, the average PCB intakes were 180 and 364 mg for females fed the 2.5- and 5.0-ppm diets, respectively [233].

Some of the exposed females began to exhibit the characteristic skin and blood chemistry signs of PCB intoxication within 2 months. At 6 months, all exhibited these signs in varying degrees. Menstrual cycles were irregular and menstrual bleeding was excessive and prolonged [233].

The conception rates for the control females and for those fed the 2.5-ppm Aroclor diet were 12 of 12 and 8 of 8, respectively, whereas 6 of 8 females fed the 5.0-ppm Aroclor diet conceived [233]. All 12 of the control group pregnancies resulted in normal births, compared to 5 and 1, respectively, for the exposed females fed the 2.5- and 5.0-ppm diets. Three of the eight fetuses of monkeys fed the 2.5-ppm Aroclor diet were resorbed shortly after conception. Profuse uterine hemorrhaging was observed rather than the implantation bleeding which usually occurred 17 days after conception, according to the authors [233]. The two females from the group fed the 5.0-ppm diet who did not conceive initially were bred five times without success. Of the six females of this group that did become pregnant, three aborted at 46, 67, and 107 days of gestation; one fetus was resorbed, one was stillborn, and one delivered normally. The six live infants born to PCB-fed mothers had body weights that were less than

normal for the colony by one to two standard deviations. These infants began showing signs of PCB toxicity (acne, swollen eye lids, increased skin pigmentation) after nursing their mothers for less than 2 months [234]. In samples obtained from three of their mothers after the infants developed the poisoning signs, the milk contained PCBs at 0.154-0.397 ppm, and the milk fat from a fourth mother contained 16.44 ppm. Three of the infants died 44-112 days after birth.

The effects of Aroclor 1254 on reproductive performance and fetal integrity were evaluated in beagle dogs and Hormel miniature swine by FL Earl et al (written communication, 1976). Aroclor 1254 was administered orally in capsules as a solution in corn oil to 46 purebred beagle bitches once daily after the 1st day of gestation. Ten bitches were given 0.25 mg/kg/day, 16 were given 1 mg/kg/day, and 20 were given 5 mg/kg/day. Sixteen untreated bitches served as controls.

The data presented show that Aroclor 1254 at 5 mg/kg/day significantly interfered with reproductive performance and was teratogenic in the beagle. When Aroclor 1254 was administered at 0.25 and 1.0 mg/kg/day, no effects on reproduction were observed but the incidence of patent fontanelles in the offspring of the 1.0-mg/kg/day group was increased sharply. Dosage at 5.0 mg/kg/day resulted in 45.5% resorptions (4-fold increase over the control rate) and an average of only two live pups per litter. Patent fontanelles were present in 50% of the offspring.

Earl and his coworkers fed Aroclor 1254 to Hormel miniature swine in doses of 1.0 (4 sows), 10.0 (6 sows), and 30.0 (7 sows) mg/kg/day for 21 days before breeding and throughout gestation. Five sows served as controls. The 1-mg/kg/day dose produced a statistically significant

percentage of fetal resorptions (23%). Increasing the dose to 10 mg/kg/day lowered the fertility rate to 50% (80% in controls) and increasing the dose to 30 mg/kg/day lowered the fertility rate to 43%. Cleft palate and syndactyly in three feet were observed in one fetus from the group fed 10 mg/kg/day. At this dose level, there was also a significant decrease in survivability with only 71% of the offspring alive after 5 days. At the 30-mg/kg/day dose, 14 of the 15 implantations were resorbed and teratogenic abnormalities were present in 100% of the observed fetuses. Cleft palates, syndactyly, and patent fontanelles were the teratogenic effects observed. The fact that pigs are normally born with closed fontanelles increases the signficance of the teratogenic findings.

(e) Studies on Mutagenicity and Cytotoxicity

To evaluate the effects of PCBs on the testes of rats, Dikshith et al [235] administered, by oral intubation, 50 mg/kg/day of Aroclor 1254 dissolved in corn oil·to a group of 18 adult male Sprague-Dawley rats for 7 consecutive days. A control group of 18 rats received corn oil without PCBs. Three rats from each group were killed 1, 7, 15, and 30 days after the last PCB administration. The testes and epididymises were separated and weighed, as were the livers. Microscopic, histochemical (acid phosphatase), and cytogenetic examinations were performed. Rats used for cytogenetic examination were injected ip with colchicine 2 hours before being killed and the chromosomes in testicular tissues were examined.

No effects on weight or gross appearance of either the testes or epididymis were noted, nor was there evidence of atrophy or hypertrophy of the testes. The only structural changes noted in the testes of the exposed group was a proliferation of interstitial tissue cells. These cells showed

an increase in acid phosphatase activity, which, according to the authors [235], denoted a change from particulate enzyme localization in testicular interstitial cells of the controls to a disseminated, diffuse localization in the testes of the exposed group.

Cytogenetic analyses showed similar chromosomal configurations in both the control and the exposed groups. In the PCB-treated rats, some apparently sporadic abnormal chromosomes were seen in a few of the metaphase figures; chromosomal abnormalities also included breaks, exchange figures, chromatin bridges at anaphase, and chromosome fragments. The significance of these findings was unclear to the authors [235].

Green et al [236] looked for mutagenic effects in the bone marrow and spermatogonial cells of male Osborne-Mendel rats fed various doses of PCBs. Rats were given Aroclor 1242 either in single oral doses of 1,250, 2,500, or 5,000 mg/kg or in multiple oral doses of 500 mg/kg/day for 4 days. Aroclor 1254 was administered orally in daily doses of 75, 150, or 300 mg/kg for 5 days. The Aroclors were mixed with corn oil, except the 5,000-mg/kg dose of Aroclor 1242, which was given undiluted. Controls were fed uncontaminated corn oil. The animals were given Colecimid (a colechicine derivative) at 4 mg/kg 24 hours after PCB dosing was completed and they were killed 3 hours later.

Aroclor 1242 produced no statistically demonstrable evidence of chromosomal damage in bone marrow cells at doses of 1,250 or 2,500 mg/kg, or at the 500-mg/kg dose level [236]. At 5,000 mg/kg, there appeared to be an increase in the number of chromosomal abnormalities. However, since most of the changes occurred in one animal, the findings were not considered to be significant. Aroclor 1254 did not produce statistically

significant numbers of chromosomal abnormalities in bone marrow cells at any dose level tested. A statistically significant number of chromosomal abnormalities was not identified in spermatogonial cells from rats fed Aroclor 1242. Spermatogonial cells from rats given Aroclor 1254 were not examined since the results with the more acutely toxic Aroclor 1242 were negative. All observed chromosomal lesions were single chromatids. The authors [236] did not exclude the possibility of point mutations since the procedures used in this experiment would not have detected such damage.

Green et al [237] also investigated the possible induction of dominant lethality in rats given Aroclor 1242 or Aroclor 1254 by oral intubation. Except for the positive controls, results showed nonreproducible positive effects that were not related to dose or stage sensitivity. The authors concluded that the two Aroclors did not appear to be mutagenic.

Wyndham et al [116] performed the "Ames" bacterial test for mutagenicity on a variety of PCBs, using Salmonella typhimurium mutant strain TA1538. A comparison was made of the mutagenic potentials of Aroclor 1254, 2,2',5,5'-tetrachlorobiphenyl, Aroclor 1268, Aroclor 1221, and 4-chlorobiphenyl. The results showed clearly that as the degree of chlorination decreased, the mutagenic potential increased. A concentration of 100 μg of 4-chlorobiphenyl in the test medium gave over 2,000 revertant colonies/plate. The more highly chlorinated biphenyls showed very little activity as mutagens.

Hoopingarner et al [238] studied the toxicities of various Aroclors at 50 ppm in the culture medium of Chinese hamster cells, and found increasing toxicity (measured by cell population survival) with decreasing

chlorination. Aroclor 1016 appeared to be disproportionately cytotoxic, on the basis of its chlorine content, suggesting to the authors that this product contained certain toxic components in higher proportions than either Aroclor 1232 or Aroclor 1242, which it resembled chromatographically. Aroclor 1254 at 100 ppm showed no apparent effect on the chromosomal integrity of human lymphocytes in vitro.

Popper et al [239] studied the effect of hepatic microsomal biotransformation systems from control mice and from mice treated with PCBs on the mutagenic potentials of the primary carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and the secondary carcinogen dimethylnitrosamine (DMN). Hepatic microsomes were isolated from male Swiss-Webster mice that had been given 500 mg/kg of Aroclor 1254 4 days earlier, and from untreated control mice. The mutagenicities of MNNG and DMN were assayed by a bacterial auxotroph reversion test using Bacillus subtilis.

Microsomes prepared from the livers of the PCB-treated mice had 2-3 times as much cytochrome P-450 activity as the controls [239]. DMN added directly to the cultures was not mutagenic at concentrations as high as 300 mM. However DMN was converted to a mutagen when exposed to such microsomes in the presence of an NADPH-generating system. When MNNG was incubated with isolated microsomes, its mutagenic activity was reduced. The authors concluded that isolated hepatic microsomes can modify the biologic activities of mutagens and that the hepatic microsomal biotransformation system could play a key role in chemical mutagenesis.

(f) Studies on Tumorigenesis

Kimbrough et al [218] distributed 400, 21- to 26-day-old weanling, Sherman strain, "COBS" female rats randomly into two equal-size groups.

Half of the rats were fed a plain commercial laboratory chow. The other 200 were fed laboratory chow containing 100 ppm of Aroclor 1260. The dosed group of rats was fed the PCB diet until 6 weeks before they were killed at the age of 23 months. Autopsies were performed on these rats as well as those that died before the exposures ended.

Microscopic examination was completed on 184 dosed animals and 173 controls. From a few to multiple elevated tan nodules were found on the liver surfaces of 170 PCB-fed rats. The nodules varied from 0.1 to several cm in diameter. Only one such abnormality was found in the controls. Of the tumors, 26 of those in exposed animals and the 1 from the control animal were hepatocarcinomic; the other 144 experimental animals had hepatocellular nodules that were described as characteristic of neoplastic nodules or synomously, hyperplastic nodules. The authors [218] said "neoplastic nodules are part of the spectrum of response to hepatocarcinogens and must be included in the evaluation of tumorigenesis." Tumors identified and analyzed from other organs had no unusual features; no apparent differences in incidence were observed between the experimental and control groups [218].

Evidence of PCB-induced hepatocarcinomas was reported by a group of Japanese investigators in 1972 [240] and in 1973 [241]. Nine groups of 12 male dd mice were fed a commercial stock diet supplemented with 100, 250, or 500 ppm of Kanechlors 300, 400, or 500. A control group of eight mice was fed the stock diet. All mice were given water ad libitum. After 32 weeks, the animals were starved for 18 hours, killed, and examined macroscopically for tumors. Seven of the 12 mice fed Kanechlor 500 at 500 ppm developed hyperplastic liver nodules and five had well-developed

hepatocellular carcinomas. None of the mice fed Kanechlor 500 at lower concentrations or Kanechlor 400 or 300 at any concentration developed hepatocarcinomas.

In 1973, Kimura and Baba [242] described neoplastic changes in the livers of rats exposed to Kanechlor 400. Thirty 10-week-old rats were separated into an experimental group of 10 males and 10 females and a control group of 5 males and 5 females. The experimental group was fed a diet supplemented with olive oil containing various concentrations of Kanechlor 400. The control group received an olive oil-supplemented diet. The diet of the experimental group of rats contained 38.5 ppm of Kanechlor 400 for the first 4 weeks, followed by 77 ppm for 8 weeks, 154 ppm for 3 weeks, 308 ppm for 3 weeks, and 616 ppm for 8 weeks. Body weights were checked weekly and showed rapid decreases during the 616-ppm feeding period; for this reason the Kanechlor 400 content of the diet was reduced to 462 ppm for the next 32 weeks. The total feeding period for the experimental and control animals was about 58 weeks (400 days).

All rats that ingested total doses of more than 700 mg of Kanechlor 400 developed hypertrophy of the liver [242]. Pale brown nodules were present on the parietal and cut surfaces of the livers of female rats that had ingested more than 1,200 mg of PCBs. However, none of the male rats had such hepatic nodules, despite having ingested equal or greater amounts of Kanechlor 400. On microscopic examination, the livers of female rats were observed to have fatty degeneration and multiple adenomatous nodules that appeared to be benign neoplastic lesions. Males did not develop such neoplastic lesions; however, their livers did exhibit fatty degeneration. Lung abscesses, pneumonia, splenic atrophy, and intracranial abscesses were

found frequently in the experimental group. The authors [242] thought that resistance to infection was lowered in the rats fed Kanechlor 400.

A formal rulemaking hearing was convened August 20, 1976 by the US Environmental Protection Agency on its proposed toxic pollutant water effluent standards for PCBs (Federal Register 42:6532-55, February 2, 1977). Part of the testimony concerned comparative studies in which groups of 100 male and female Charles River rats were fed 1, 10, and 100 ppm of Aroclors 1242, 1254, or 1260 for 24 months. The survival of the rats was poor and the experiment was said to have been inconsistently reported. A reevaluation of the pathology slides revealed the occurrence of liver tumors (hepatomas and cholangiohepatomas) in the rats fed any one of the three Aroclor products. The incidence of liver tumors in the rats dosed at 100 ppm was 3/20 for Aroclor 1242, 6/27 for Aroclor 1254, and 7/27 for Aroclor 1260, compared with 0/20 for the controls. In addition to the tumors there was a high frequency of nodular hyperplasia. The incidence of nodular hyperplasia at 100 ppm was 8/20 for Aroclor 1242, 13/27 for Aroclor 1254, and 7/27 for Aroclor 1260, compared with 1/23 for the controls. Nodular hyperplasia in the rats dosed at 10 ppm occurred in 2/10 for Aroclor 1242, 3/26 for Aroclor 1254, and 9/23 for Aroclor 1260, and 1/23 for the controls. The incidence of tumors of the pituitary gland also was elevated in each treated group.

Correlation of Exposure and Effect

(a) Absorption, Metabolism, Excretion, and Body Burdens

It has been demonstrated in experimental animals that PCBs can be absorbed into the blood from inhaled air [97] and from the digestive tract

[50,51,95,96]. Qualitatively, the effects in experimental animals from absorption by either route were similar [98,99,202-206]. When PCBs were applied to the skin, certain effects, such as those on the liver, were similar to those obtained when PCBs were ingested or inhaled [205-208]. Thus, it appears that PCBs were also absorbed by this route.

In experimental animals, most PCB isomers were metabolized in varying degrees to more polar compounds such as hydroxylated derivatives [41,48,49,51-56,58-81,85,87]. The more highly chlorinated isomers were metabolized slowly and accumulate in the tissues [41,50,51,55,61-64,66,82-84,96,103,107-110]. The hydroxylated derivatives were excreted in the bile, urine, and milk [41,48,50-56,58-61,63-66,69,71-80,85]. Unmetabolized compounds were excreted in feces, milk, and hair [41-44,50,51,53-56,100-102], and traces were found in urine [42,51-55,57-62,103-105].

In man, the metabolism of PCBs has not been studied. However, finding that PCB isomers that are difficult to metabolize become concentrated in residual PCBs of tissues from the general population, from persons who have ingested PCBs, and from those with occupational exposure suggests that man metabolizes, eliminates, and stores the compounds in ways similar to those of animals [7,36,136,168,171-175]. Determinations of PCBs or their metabolites in urine or feces of humans have not been reported. PCBs have been found in human hair [102,142]. Excretion in human milk of PCBs with compositions similar to those in blood, has been reported in association with general environmental exposures and following ingestion and occupational exposures [143,144,168,192]. These findings of PCBs in human hair and milk also suggest metabolism and excretion similar to those of animals.

Because PCBs are widespread in the environment, detectable concentrations have been found in tissues and body fluids of a substantial portion of the US population [36,102,134,135,137,140,144]. Detectable concentrations have been found in up to 62% of blood serum samples, with concentrations ranging up to 30 ppb [137]. In emaciated patients, blood PCB concentrations as high as 100 ppb have been found [140]. PCB concentrations in about three-fourths of adipose tissue samples from the general population were <1 ppm, and the remainder were mostly in the range of 1-2 ppm [36,136]. Usually the PCB isomers found in blood and adipose tissue have been penta-, hexa-, and heptachlorobiphenyls [7,135,136], and a high correlation was found between the concentrations in these two tissues [141]. PCB isomers in cord blood were qualitatively identical to those in maternal blood [138]. PCB isomers excreted in human milk were similar or identical to those in blood [168], and the concentrations in milk were positively correlated with adipose tissue concentrations [143]. In human milk samples from the general US population, PCB concentrations up to 13 ppm (fat basis) have been reported (EP Savage, written communication, February 1977).

These concentrations of PCBs in blood, adipose tissue, and milk of the general US population arise from dietary intakes estimated to be of the order of 10-20 μg/day [46] and from inhalation of air that may contain up to 100 ng/cu m [26,38].

(b) Irritation Effects and Chloracne

The first reports of adverse effects of PCBs on workers included chloracne [120,123,130], digestive disturbances [123], irritation of mucous membranes [120,123,130], and impotence [123]. The exposures associated

with these effects were not well-defined either qualitatively or quantitatively. When PCBs were first being manufactured, (in mostly open systems) the exposures were to benzene and unidentified intermediates in addition to PCBs [120]. Jones and Alden [120] and Schwartz [123] did not report exposure concentrations in their studies. However, Drinker et al [125] indicated that average concentrations of PCBs ranged from about 0.5 to 1.5 mg/cu m in 30 factories, which may have included the factories studied by Schwartz [123]. On the basis of these reports [120,123,125], it would appear that chloracne and some other systemic effects occurred with early PCB preparations when their average concentrations in workroom air were below 1.5 mg/cu m. Elkins [130] found that airborne PCB concentrations approaching 10 mg/cu m in capacitor-impregnating operations in Massachusetts were "unbearably" irritating to the workers, but there were no apparent toxic effects with concentrations that averaged up to 5.8 mg/cu m. Elkins [130] did not describe the processes or the PCBs used.

Puccinelli [186] found that when capacitors were filled with Aroclor 1254 heated to 70-80 C, PCB concentrations of about 5-7 mg/cu m resulted. The workers studied by Puccinelli [186] developed chloracne after 4-8 months of exposure. In studying similar processes, Hofmann and Meneghini [187] found that chloracne developed after 2.5-4 months of exposure. These investigators [187] also described the development of areas of brown skin on the foreheads of workers. They did not describe the PCBs used or the exposure concentrations. Reports of the development of chloracne in Americans, who worked in operations which used heated PCBs, appeared in 1964 and 1969 [188,189]. In one of the processes [188], an Aroclor with a chlorine content of 65% and containing both PCBs and polychlorinated

terphenyls was used; the PCB used in the other process [189] was not described, but extensive skin exposure from wearing PCB-soiled clothing and from immersion of unprotected hands into the PCB mixture was described.

A case of chloracne that developed in an Australian worker after exposure to Aroclor 1242 at concentrations of 1-2 mg/cu m was reported by Ouw et al [196]. The PCB preparation was heated during a capacitor-filling process. Complaints of process workers included irritation of the face, eyes, and skin. Eczematous rashes were also found on their hands and legs.

In the reports of chloracne development in occupations involving PCBs (where environmental concentrations of PCBs were measured) [125,130,186-189,196], the sampling and analytical methods did not distinguish between vapor and particulate forms of the PCBs. However, a common factor in many of these reports [186-189,196] was the use of heat in the process which would tend to generate PCB vapors.

In their study of capacitor and PCB manufacturing plants in Japan, Hasegawa et al [191] found that concentrations of PCB vapors (or particles <0.1 μm in diameter) exceeded the concentrations of PCBs in particles >0.1 μm in diameter. The compositions of PCBs in the particulate were similar to those in the PCB preparations in use, but the less highly chlorinated compounds were concentrated in the vapors. In this study, where PCB vapors ranged from 0.026 to 0.965 mg/cu m and PCBs in the larger airborne particulates ranged from 0.19 to 0.650 mg/cu m (maximum total PCBs of 1.6 mg/cu m), dermal ailments included a brown chromodermatosis of the hands and chloracne. The workers had been exposed to PCBs from <1 to 20 years.

Meigs et al [190] described a process in which chloracne developed where the exposures had to have been to PCB vapors. The PCB which was used

as a heat-exchange medium was not described, but PCB concentrations were reported to be about 0.1 mg/cu m in the workroom air where the workers were exposed. Under these conditions, chloracne developed after a minimum of 5 months of exposure.

Chloracne has also been studied in connection with determinations of PCB concentrations in blood. Hara et al [192,193] found whole blood PCB concentrations of 7 to 300 ppb in Japanese workers engaged in capacitor filling. About 40% of these workers had chloracne and 13% had irritation of the skin. Kitamura et al [194] found PCBs at 320-820 ppb in blood samples from Japanese workers engaged in capacitor manufacture. Skin ailments, including chloracne, were found in 10 of 13 workers studied. Inoue et al [195] found chloracne in a man exposed to PCBs in a silk-glossing factory who had a concentration of about 200 ppb of PCBs in his blood, but they [195] found what were described as relatively mild skin abnormalities in 28 other workers engaged in similar processes who had blood PCB concentrations mostly under 100 ppb (2 had concentrations >100 ppb). Ouw et al [196] concluded from their study of capacitor workers that no adverse effects were found in workers with blood PCB concentrations <200 ppb.

Studies of PCB-exposed workers where chloracne has not been found include those of Levy et al [197], Karppanen and Kolho [198], and Bumgarner et al [199]. In the study by Karppanen and Kolho [198], workers who had been exposed to PCBs for 4 years in a capacitor-impregnating operation had PCB concentrations of 74-1,000 ppb in samples of their blood. The environmental exposure concentrations of PCBs were reported to meet "internationally accepted standards." Other factors of the work situation,

such as the skin protection used, were not reported. In the study by Levy et al [197], workers who had been exposed to PCBs for 2.5-18 years had concentrations of PCBs in samples of their blood ranging from 36 to 286 ppb. PCB exposure concentrations at the time of the study were 0.013-0.264 mg/cu m. Although some of the workers complained of throat or eye irritation, and some skin rashes were found, no cases of chloracne were seen. From the description of the work situation, it is unlikely that skin exposure to liquid or solid PCBs was an important factor in the total exposure of these workers. In their study of refuse workers, Bumgarner et al [199] found blood PCB concentrations to be 4-14 ppb. One possible source of exposure of these workers was airborne incinerator effluents, and it is unlikely that additional exposure from skin contact occurred.

Although skin exposure was unlikely in these two reports [197,199] where chloracne was not found, skin exposure is not necessary for chloracne to develop. Chloracne has been observed in Japanese people estimated to have ingested total PCB doses of 0.3-4 g [146,164-167], and chloracne-like lesions have been produced in experimental rhesus monkeys fed PCBs at 3 ppm in their diets [222].

Thus, although eliminating exposure of the skin through engineering controls and using appropriate protective clothing and work practices will reduce the total absorption of PCBs, these practices will not necessarily eliminate chloracne. The data indicate that chloracne may occur with exposures to PCB vapor concentrations as low as 0.1 mg/cu m for several months, and with PCB concentrations in the blood of about 200 ppb.

(c) Effects Referable to the Liver

Some clinical and autopsy findings in Yusho, the disease that occurred after ingestion of PCB-contaminated rice oil by humans, were indicative of liver injury [155-158,179-181]. These findings included changes in liver cell anatomy considered consistent with microsomal enzyme stimulation and increased SAP activity. Effects that persisted for several years included decreased concentrations of iron and bilirubin in the serum [155,179] and increased serum concentrations of triglycerides. The latter were found to increase with residual concentrations of PCBs in the serum [180].

The maximum amounts of PCBs consumed by individuals manifesting these effects were estimated to have been of the order of 3-4 g total intake over several months [165]. For two Yusho patients a daily PCB consumption of 67 μg/kg for 3 months was estimated [167]. The maximum intake would have been of the order of 50 ppm in the diet or 0.5-1 mg/kg/day. For comparative purposes, absorption of PCBs from inhalation during maximum occupational exposures that have been reported (10 mg/cu m) probably did not exceed 0.15 mg/kg/day.

The extent to which PCB consumption was responsible for the effects on the liver is not known since the contaminated oil also contained PCDFs [20,160] in quantities that could have resulted in a maximum PCDF consumption of 20-50 mg. Autopsy findings 1-3 years after the poisonings indicated unusually high concentrations of PCDFs (0.3-2.5 μg/g) relative to PCBs (3.5-5.6 μg/g) in liver fat [160].

Although most animal feeding experiments have been conducted with dietary levels of PCBs that are much higher than those ingested by Yusho

patients, others have been conducted with dietary PCB levels that seem to confirm the Yusho findings. Experiments have been reported with various commercial PCB mixtures (Aroclors 1242, 1254, 1260, and Clophen A60) added to the diet at 1-10 ppm [101,209-212,215,228,233]. These experiments demonstrated increased liver weights at all concentrations, dose dependent stimulation of microsomal enzyme activities, detectable proliferation of the SER at concentrations of 10-100 ppm, and other microscopic changes including enlarged hepatocytes, hepatocytic vacuolization, and, with prolonged exposures, development of adenofibrosis.

At high PCB concentrations (>1.5 mg/cu m), inhalation experiments with animals have demonstrated increased liver weights [98,99,202,203]. Microscopic changes similar to those seen from PCB ingestion were found in livers of rats after inhalation of Aroclor 1254 at 1.5 mg/cu m or more [203], and from other commercial preparations (Decachlorodiphenyl and Solvol) at much higher concentrations (>4 mg/cu m) [99,202].

These data from the Yusho poisonings and from animal experiments indicate that hepatic effects seen in human are similar to those seen in animals. They also indicate that some commercial preparations may be less severe liver poisons than others, but the data are not definitive in this regard. The ingestion experiments demonstrated increased liver weights from feeding PCBs at 1 ppm, and increasing evidence of liver injury as dietary PCB levels were increased. A dietary level of 1 ppm may be considered roughly equivalent to a daily intake of the order of 0.01-0.02 mg/kg, which would be somewhat similar to the intake from inhaling PCBs at 1 mg/cu m.

Several reports of occupational exposures to PCBs have involved the liver in various ways [123,125,127,186,190,191,197,198,200].

In the earliest reports of PCB toxicity [123,135,127], the PCBs were mixed with chlorinated naphthalenes. The mixtures caused many cases of chloracne and, in some cases, jaundice. Liver cirrhosis with superimposed yellow atrophy was found in at least one fatality [125]. Similar findings have not been reported where exposures were to only PCBs in the absence of chlorinated naphthalenes.

Mention of nausea and digestive disturbances in some reports [123,197] may have indicated liver injury. In the study by Schwartz [123], neither liver function test findings nor exposure concentrations were reported. In the Levy et al report [197], liver function tests (SAP, SGOT, SGPT, total bilirubin) performed at the time of the study did not indicate current liver injury. The workers had been exposed to unidentified PCBs at 0.013-0.264 mg/cu m, and blood PCB concentrations were 36-286 ppb. Even though there were no findings of current liver injury, these investigators' [197] examination of past medical records showed occasional findings indicative of slight liver injury (elevated serum enzymes, triglycerides, and uric acid). A report of a worker who was removed from further exposure after experiencing nausea on exposure to an askarel containing Aroclor 1254 was not accompanied by liver function test results or exposure concentration data (In the Matter of General Electric Company, File No. 2833, New York State Department of Environmental Conservation, 1975).

More conclusive studies of liver function were reported by Meigs et al [190], Hasegawa et al [191], Hara et al [192,193], and Ouw et al [196]. Of the workers exposed to vapors of an undefined PCB at 0.1 mg/cu m in the

study by Meigs et al [190], liver function tests were performed on seven. Findings in six of the workers were normal and, in the other one, cephalin flocculation and thymol turbidity tests were on the borderline of abnormality. Hasegawa et al [191] found some changes in liver function tests (increased SGOT, SGPT, and SAP, and decreased serum cholinesterase and lipids) of 99 workers exposed to Kanechlors but the investigators considered that only the decreased serum lipids were significant. PCB exposure concentrations at the time of the study were 0.045-1.6 mg/cu m. The workers had exposure histories of <1 to 20 years. PCB concentrations in blood samples of the workers averaged 370 ppb.

Hara et al [192] did not find evidence of a correlation of serum triglyceride concentrations with blood PCB concentrations during the first year after the work with PCBs was discontinued. However, when the same workers were studied 2 years after stopping work with PCBs, the proportion of workers with elevated serum triglycerides increased as residual blood PCB levels increased [193]. The effect was particularly prominent in workers with residual blood PCB concentrations >50 ppb. In a study of 34 workers exposed to Aroclor 1242 in capacitor manufacture, Ouw et al [196] found individual abnormalities in SGPT, SAP, and serum bilirubin, but average values for the group were normal. Bromsulphalein retention tests were elevated in four workers. Ouw et al [196] considered that there were no adverse responses in workers with blood PCB concentrations below 200 ppb. The PCB exposure concentrations were 0.32-1.44 mg/cu m.

No evidence of impaired liver function was found in other studies [186,194,195,198] where PCB exposures were reported as: Aroclor 1254 at 5-7 mg/cu m for 2-4 years [186]; Kanechlors at >0.25 mg/cu m [195]; and

unidentified PCBs at <1 mg/cu m for 4 years [198].

Evidence relating to impaired liver function from occupational exposures to PCBs is generally consistent with findings in Yusho patients and in animal studies that indicated some effects could be expected from PCB intakes equivalent to PCB inhalation at about 1 mg/cu m. The occupational exposure studies show occasional evidence of liver injury in workers exposed at concentrations of 0.013-0.264 mg/cu m [197], 0.1 mg/cu m [190], 0.045-1.6 mg/cu m [191], and 0.32-1.44 mg/cu m [196]. Whether the observed effects occurred with exposures at the lower end of all these ranges is not known.

The occupational exposure studies also showed delayed recovery of normal serum triglycerides after exposures to PCBs stopped [193]. The elevated serum triglycerides were related to the higher residual blood PCB concentrations, and these in turn were related to the years of exposure. Residual PCBs have repeatedly been shown to be the more highly chlorinated isomers and those metabolized with greater difficulty [7,36,51,83,103,110,135,136].

Summaries of effects of PCBs on humans and animals are presented in Tables III-9 and III-10, respectively.

Carcinogencity, Mutagenicity, Teratogenicity, and Effects on Reproduction

(a) Carcinogenicity

There is extensive evidence for formation of arene oxide intermediates during the metabolism of PCBs by several species including rhesus monkeys [51,68,69,74,75,81,115,116,118]. Arene oxide formation was also proposed as a plausible intermediate in the metabolism of the slowly

metabolized 2,2',4,4',5,5'-hexachlorobiphenyl [70,79]. Binding PCB metabolites to nuclear components of liver cells has been demonstrated on administration of PCBs to monkeys [51], and to rats [81], and by in vitro experiments [51,82,86,116,117]. This information is sufficient to arouse suspicions that PCBs could have carcinogenic potential in humans.

Hepatomas were produced in 170 of 184 examined female rats fed Aroclor 1260 at 100 ppm from 3-4 to 23 months of age [218], and in 9 of 22 BALB/cj male mice fed Aroclor 1254 at 300 ppm for 11 months [221]. The tumors found in the rats included 26 hepatocarcinomas and 144 neoplastic nodules. Only one liver tumor, a hepatocarcinoma, was found in 173 control rats.

In another report (Federal Register 42:6532-55, February 2, 1977), dose-related incidences of liver tumors were reported in rats fed Aroclors 1242, 1254, or 1260 at 1, 10, and 100 ppm in the diet for 24 months. No liver tumors were reported in rats fed the diets containing PCBs at 1 ppm. Only one tumor, a hyperplastic nodule (neoplastic nodule), was found in 23 controls. Neoplastic nodules were found in the livers of rats fed the diet containing 10 ppm of PCBs (2/10, 3/26, and 9/23 for Aroclor 1242, 1254, and 1260, respectively). Higher tumor incidences were found when diets containing PCBs at 100 ppm were fed (11/20, 19/27, and 14/27 for Aroclors 1242, 1254, and 1260, respectively) and the tumors included hepatomas and cholangiohepatomas (3, 6, and 7 for Aroclors 1242, 1254 and 1260, respectively). In this experiment the incidence of pituitary tumors was high in PCB-fed rats.

In a study sponsored by the National Cancer Institute, Aroclor 1254 was fed to groups of 24 rats at 25, 50, and 100 ppm in the diet. No liver

tumors were found in the controls, in the experimental females at any dose level, or in experimental males fed PCBs at 25 ppm. However, hepatocellular carcinomas were found in a male rat fed Aroclor 1254 at 50 ppm and in two males fed the PCB at 100 ppm. The data also indicated that the incidence of leukemias in males was dose-related (3/24 in controls; 8/24 in rats fed 100 ppm).

Kanechlors have also been found to produce liver tumors when fed to mice and rats [240,241,242]. Of 12 mice fed Kanechlor 500 at 500 ppm for 32 weeks, 7 developed neoplastic liver nodules and 5 had well-developed hepatocellular carcinomas [240,241]. In this experiment which had only 12 mice/group and continued for only 32 weeks, liver tumors were not found with Kanechlors 300 and 400 or with lower dose levels of Kanechlor 500. However in another experiment [242], Kanechlor 400 fed to 10 male and 10 female rats for 58 weeks did produce liver tumors in the females. In this experiment the amounts fed (38-616 ppm) varied from time to time, but were in the 308-616 ppm range for the last 43 weeks.

These findings from animal studies indicated to NIOSH that PCBs have potential carcinogenic activity in humans. They indicate, but do not conclusively demonstrate, with the dietary levels used, that the less highly chlorinated mixtures (Aroclor 1242 and Kanechlors 300 and 400) have less carcinogenic potential than the more highly chlorinated mixtures (Aroclors 1254 and 1260 and Kanechlor 500). However, all PCB mixtures adequately tested in rats and mice have shown carcinogenic activity. The intakes of PCBs at the lowest dietary level that has produced tumors in rats (10 ppm) would be somewhat comparable to intakes from occupational exposures at 5-10 mg/cu m. However PCBs are slowly eliminated from the

body and the higher chlorinated compounds may accumulate in the body for years. Thus animal experiments that are limited to 2 years by the life span of the animals may not be informative relative to workers exposed for up to 45 years.

In humans, there are no adequate studies to confirm or deny carcinogenicity although preliminary data suggest that among Yusho patients, deaths due to cancers exceed expectations [160,161] and preliminary studies of two occupationally exposed groups in the US indicate that the occurrence of certain cancers may be excessive (HA Sinclaire, written communication, June 1976; G Roush, written communication, September 1976). However, the two reports are not consistent as to the types of cancers found to occur more frequently than expected.

(b) Mutagenicity

Several PCBs and PCB mixtures, including the 4- and the 2,2',5,5'-isomers and Aroclors 1221, 1254, and 1260, were subjected to the "Ames" test for mutagenicity [116]. Although 4-chlorobiphenyl had mutagenic activity in this test, the more highly chlorinated PCBs showed very little activity. Aroclor 1254 did not cause significant chromosomal changes in the testes of rats after it was administered for 7 days at 50 mg/kg/day [235]. In another experiment [236], neither Aroclor 1254 administered at 300 mg/kg/day for 5 days nor Aroclor 1242 administered at 500 mg/kg/day for 4 days produced chromosomal aberrations in spermatagonial or bone marrow cells of rats. These mixtures also did not produce any evidence of dominant lethal mutations in rats [237]. Although PCBs have little mutagenic potential, they may alter the mutagenicity and carcinogenicity of other compounds by stimulating microsomal enzyme activities [239].

(c) Teratogenicity

PCBs have been found in embryonic and fetal tissues of humans [139,168] and experimental animals [101] after introduction of PCBs into the maternal body, demonstrating that the potential for direct teratogenic effects exists. Several experiments have been conducted with rats [101,228], rabbits [229], monkeys [232], and dogs and pigs (FL Earl et al, written communication, 1976) that are relevent to a discussion of PCB teratogenicity. In some of these experiments, the PCBs were administered by gavage and doses were reported in mg/kg; in other experiments, the PCBs were reported as ppm fed in the diet. For purposes of relative comparison, 50 ppm in the diet can be equated to 1 mg/kg/day. This is the order of magnitude of the maximum rate of PCB intake by Yusho patients. Animal experiments have used PCBs in dietary-equivalent amounts of 1-2,500 ppm. In most experiments with PCBs administered in amounts equivalent to dietary levels of 100 ppm or more, fetotoxicity (resorptions, absortions) has been such that teratogenic effects may have been masked [101,228,229]. In the two-generation feeding study of rats by Linder et al [228], no terata were reported. This study covered Aroclor 1254 in the concentration range of 1-100 ppm and Aroclor 1260 in the range of 5-100 ppm. Although terata were not reported, Aroclor 1254 concentrations of 20-100 ppm resulted in reduced litter sizes. In rhesus monkeys [232], feeding Aroclor 1248 at 2.5 and 5 ppm caused abortions in some cases and lower than normal birth weights, but no terata were reported. In dogs, terata were not found in pups born from dams fed the equivalent of 12 ppm in the diet, but were present when 48- or 200-ppm equivalents were fed. Sows fed the equivalent of 50 ppm in the same experiment had high rates of resorptions and, at 10-30 times this

level, terata were definitely present in the piglets (FL Earl et al, written communication, 1976).

Although there were retarded intrauterine growth and signs of PCB toxicity in Yusho babies at birth, no terata were reported [149,159,176,182,183]. A normal baby was born to a woman exposed to PCBs in her work. The PCB exposure concentrations were not reported but the PCB concentration in her blood was 25 ppb at the time the baby was born.

These studies indicate that PCBs have teratogenic potential for humans. However, the terata observed in animals occurred at levels at or above doses equivalent to the maximum doses of the Yusho patients and at intake rates 3-4 times greater than intakes expected from inhalation at maximum reported occupational exposures.

(d) Other Effects on Reproduction

Feeding rats Aroclor 1254 at 5 ppm or Aroclor 1260 at 100 ppm had no effects on reproduction over two generations [228]. At higher dietary levels, reproductive effects included poor mating performances, fewer litters, reduced litter size, and high postnatal death rates in the litters. In rabbits, Aroclors 1221 and 1254 were not fetotoxic when administered only during gestation in amounts equivalent to dietary levels of about 50-500 ppm [229]. However, when administered during gestation at higher levels (600-2,500 ppm dietary equivalent), Aroclor 1254 caused resorptions, abortions, maternal death, and, in two fetuses, asymmetric skulls [230]. Delaying administration of PCBs until after the first week of pregnancy did not eliminate the effects.

Feeding rhesus monkeys Aroclor 1248 at 25 ppm for 2 months resulted in a high degree of infertility that persisted for at least 8 months after

the last ingestion of PCBs [232]. In another experiment, rhesus monkeys fed Aroclor 1248 at 2.5 and 5 ppm for 6 months had irregular menstrual cycles with excessive and prolonged bleeding [233]. Even though these monkeys conceived well on mating, resorptions and abortions were frequent, and infertility was common in subsequent matings [233]. Nursing infants developed chloracne-like signs within 2 months and infants frequently died during the nursing period. The milk contained PCBs at 0.154-0.397 ppm [234].

There are no reports of infertility or abortions attributed to human PCB consumption or exposure. There are reports of undesirable effects in children born to mothers exposed to PCBs in the diet and of undesirable effects developing from nursing such mothers.

Babies born from mothers with Yusho were often dark colored and developed signs of Yusho after nursing [176,182,183]. In at least one case, Yusho developed in a baby who was only exposed to PCBs by nursing [182]. Concentrations of PCBs in milk were not determined at the time of the Yusho poisoning. A milk sample obtained after birth of a normal baby from a woman exposed to PCBs at work contained PCBs at 0.25 ppm, and nursing was discontinued [195]. The woman's exposure history and time of last exposure were not given, but PCBs were present in her blood at 24 ppb. Based on the findings in monkeys [234] the decision to not nurse the baby seems entirely justified.

TABLE III-9

EFFECTS OF PCBs ON HUMANS

PCB Intake		Exposure Index			
Source	Duration	Environmental, Amount/kind	Blood (ppb)	Effects	Ref.
Ingestion	Up to 8 mon	0.3-4g Kanechlor 400	>50	Yusho	145-185
Occupational	Not known	10 mg/cu m	-	Unbearable irritation	130
"	4-8 mon	5-7 mg/cu m Aroclor 1254	-	Chloracne, no liver injury	186
"	2.5-4 mon	Not reported	-	Chloracne, hyperpigmentation	187
"	<1-20 yr	0.2-1.6 mg/cu m Kanechlors	370, ave.	Chloracne, hyperpigmentation, liver injury	191
"	2.5 yr ave.	Not reported Kanechlors	820, ave.	Chloracne, no liver injury	194
"	Not reported	>0.25 mg/cu m Not reported	130-520	Chloracne, liver injury	195
"	2.5-18 yr	0.013-0.27 mg/cu m Not reported	36-286	Irritation, liver injury	197
"	14 mon ave.	0.1 mg/cu m Not reported	-	Chloracne, liver injury	190
"	"	Not reported refuse workers	<1-14	No effects	199

TABLE III-9 (CONTINUED)

EFFECTS OF PCBs ON HUMANS

PCB Intake		Exposure Index			
Source	Duration	Environmental, Amount/kind	Blood (ppb)	Effects	Ref.
Occupational	2-23 yr	0.32-1.44 mg/cu m Aroclor 1242	>200	Chloracne, liver injury	196
"	1-23 yr	0.32-1.44 mg/cu m Aroclor 1242	<200	No effects	196
"	Up to 15 yr	Not reported Kanechlors	7-300	Chloracne, elevated triglycerides	192, 193
"	Not reported	Not reported Kanechlors	24 10-100	Normal baby Mild chloracne	195
"	4 years	(>1 mg/cu m) Aroclor 1242	74-1,900	No effects	198
General environment	Continuous	Air, 1-100 Food, 10-20 µg/d	>1-30	No effects	137-141

TABLE III-10

EFFECTS OF PCB INHALATION ON ANIMALS

Material	Exposure Conditions mg/cu m	Duration	Species	Effects	Ref.
Pydraul A 200	30,000	2 hr	Rats	PCB in liver, 70 ppm	97
Solvol	10,000	3 hr	"	100% mortality, liver necrosis	202
"	250-500	8-69 3-hr exposures	"	Liver necrosis, hyperplasia of Kupffer cells	202
Decachlorodiphenyl	2,500	6 hr	"	Irritation, no gross effects	98
"	800	6 hr/d, 5 d/wk, 4 wk	"	Irritation, increased liver weights, microscopic liver changes blood cell changes	99
"	4 and 80	"	"	No irritation, increased liver weights in females	99
Aroclor 1242	8.6	7 hr/d, 5 d/wk, 3 wk	Mouse, rat, cat, guinea pig, rabbit	None	203
"	6.8	7 hr/d, 5 d/wk, 17 wk	"	"	203
"	1.9	7 hr/d, 5 d/wk, 31 wk	"	"	203
Aroclor 1254	5.4	7 hr/d, 5 d/wk, 17 wk	"	Microscopic liver changes	203
"	1.5	7 hr/d, 5 d/wk, 31 wk	"	Microscopic liver changes; reversible	203