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TOXICITY OF CHLORINATED BIPHENYLS

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Despite the fact that polychlorinated biphenyls (PCBs) have been available commercially for 40 years, it is only within the last 5 years that they have been recognized to be of environmental and potential toxicologic concern.

PCBs are produced by a comparatively small number of manufacturers in the USA, France, Germany, and Japan and marketed under a number of commerical trade names, e.g. Aroclor, Clophen, Phenoclor, and Kaneclor.

The series of Aroclors (Monsanto) are marketed under various numbers and consist of mixtures of chlorinated biphenyls and terphenyls. The first two digits represent the molecular type: 12-chlorinated biphenyls; 25 and 44-blends of chlorinated biphenyls and chlorinated terphenyls (75% biphenyl and 60% biphenyl, respectively); 54-chlorinated terphenyls. The last two digits give the weight percent of chlorine, e.g. Aroclor 1242 is a chlorinated biphenyl containing 42% chlorine.

The PCBs have been employed in a broad spectrum of applications because of their chemical stability, low volatility, high dielectric content, nonflammability, and general compatability with chlorinated hydrocarbons. The major areas of utility include: heat exchanger and dielectric fluids, hydraulic and lubricating fluids, plasticizers for plastics and coatings, ingredients of caulking compounds, printing inks, paints, adhesives, and carbonless duplicating paper, flame retardants, and extender for pesticides.

The rates and routes of transport of the PCBs in the environment (1), and their accumulation in ecosystem (2–16), have been cited. Salient features of the chemical (3, 4, 7, 11, 14–17), analytical (3–18), biological (3, 7, 9, 14–20, 28, 30–38), aspects of the PCBs as well as their occurrence in human diets (2–4, 14, 15, 39–42), and tissue (14–16, 33–38, 43–47), have all been reported.

The major objective of this review is to highlight the status of the toxicologic, carcinogenic, teratogenic, and mutagenic aspects of the PCBs that are of greatest relevance to man.

Compared to the chlorinated hydrocarbon pesticides, definitive aspects of acute, subacute, and chronic toxicity still remain rather poorly known. The toxicological

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characterization of PCBs is confounded by the fact that the commercial products are mixtures of isomers, and significant traces of chlorinated dibenzofurans and naphthalenes have been found in several preparations (e.g. Clophen A-60 and Phenoclor Dp-6) (48).

ANIMAL TOXICITY

Early studies of acute oral, dermal, and vapor pressure of the PCBs have involved in many cases mixtures or compounds of undefined specifications and hence have been rather difficult to interpret unambigously.

a. Acute and Subacute Toxicity

Table 1 summarizes measurements of the oral and dermal toxicity of seven Aroclor mixtures to rats and rabbits respectively, and indicates that the PCBs are of a low order of toxicity when administered as a single dose. These results suggest that while the oral toxicity to rats decreases with increasing chlorination, there is no apparent trend of toxicity with chlorination in the data for rabbits. Miller (49), studied the toxicity of a PCB mixture equivalent to Aroclor 1242 and indicated that the guinea pig was the most sensitive of the three species, followed by the rabbit and the rat in that order.

Table 1 Toxicity of Aroclors*

	Aroclors										
	1221	1232	1242	1248	1260	1262	1268	4465	5442	5460	2565
Oral LD_{50} mg/kg (rats)	3980[a]	4470[a]	6650[a]	11,000[a]	10,000[b]	11,300[b]	10,900[b]	16,000[b]	10,600[b]	[illegible]	[illegible]
Skin MLD mg/kg (rabbits)	>2000[b]	>1260[b]	>194[a]	>794[a]	>1260[b]	>1260[b]		>2000[b]	>1260[b]	[illegible]	[illegible]
	<3169[a]	<2000[a]	<1269[a]	<1269[a]	<2000[b]	<3160[b]	<2300[c]	<3160[b]	<2000[b]		<3160

*LDA Status Report on the Chemistry and Toxicology of Polychlorinated Biphenyls (PCB) or Aroclors as of June 1, 1970 (Ref. 30)
[a]Undiluted
[b]Administered as 50% solution in corn oil
[c]Administered as 33 1/3% solution in corn oil

Analogously with the chlorinated hydrocarbon pesticides the most important effects are long-range sublethal effects. Aroclor 1254 at 1000 ppm in the diet was fatal to ¾ male rats in 43 days and it was reported by Tucker & Crabtree (50) that a total intake of 500–2000 mg/kg was the lethal level under these conditions. The single-dose oral LD_{50} for Aroclor 1260 and 1254 in rats is considerably higher (Table 1), hence the lethal effect appears to be of a highly cumulative nature.

Rehfeld et al (51) studied the subacute toxicity of Aroclor 1248 in 10-day-old chickens. The mortality after 25 days feeding at 50, 40, 30, and 10 ppm in the diet was 16/30, 4/20, 1/30, and 0/10 respectively. Only 2–4 out of 10 chicks survived on diets containing 100 and 150 ppm. Kohanawa et al (52) found Kanaachlor-400

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(~33% chlorination) to be fatal to 10/10 chicks within 5 days at a concentration of 300 ppm, while at 100 ppm there were no mortalities in 20 days. It is important to note that different batches or sources of PCBs of similar degrees of chlorination appear to vary as to potency, as reported by Vos & Koeman (33) in their studies in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. Aroclor 1260 caused only 15% mortality in chicks after 8 weeks, with an average time of death of approximately 3 weeks. The greater toxicity of the latter preparation was attributed to the presence of trace amounts of chlorinated dibenzofurans (probably tetrachloro- and pentachlorodibenzofuran) (38).

Table 2 depicts pathologic changes induced by PCB, and illustrates some interesting differences between mammals and birds. For example, the most striking findings in mammals are alterations to the liver, whereas fluid in the pericardial sac, kidney damage, and reduced spleen are found in birds. PCB-induced death in rats, rabbits, and guinea pigs is accompanied by liver lesions, including fatty infiltration, centrolobular atrophy necrosis (49, 52), and in the case of rats, by hyaline degeneration. Except for chloracne-like lesions occurring at the point of skin injection or intradermal infection, other organs in these species are not prominently affected. In birds, the most consistently observed lesions are hydropericardium and ascites as seen in the chicken (52–55), Japanese Quail (52), and bob whites (56). Other lesions in birds experimentally poisoned with PCBs include kidney damage (52–54), liver damage (52, 54), enteritis and intestinal hemorrhages (54), subcutaneous edema (52, 55), and dermatitis (53).

Nishizumi (57) studied the effects on mouse and monkey liver of chlorinated biphenyls [48% chlorine, equivalent to three to four atoms of chlorine per molecule, with a trace (0.01% of naphthalenes). Groups of 30 female mice were given a dosage level of 0.2 ml rice bran oil containing 1600 ppm or 0.5% PCB in olive oil by stomach tube each day for 4–26 weeks resulting in marked liver enlargement. (Light microscopy revealed only slight liver changes but electron microscopy disclosed marked alterations in the liver cells.) A similar study with 8 monkeys (5 cynomolgus and 3 squirrel) given chlorinated biphenyls in dosage levels of 1.4–1.6 mg/day in their diet for 40–48 days showed both liver cell enlargement and fatty degeneration. The major abnormality reported for the administration of chlorinated biphenyls to mice and monkeys was an increase in the smooth endoplasmic reticulum in the liver cells.

The administration of high doses of Aroclor 1242 to rats by oral intubation produced diarrhea, chromodacryorrhea, loss of body weight, unusual stance and gait, lack of response to pain stimuli and central nervous system depression apparently contributed to each fatality. Histopathological changes appeared only in the liver and kidneys as foci of sudanophilic vacuolation (53).

Rats given 100 mg Aroclor 1242/kg every other day for 3 weeks showed similar histopathological changes but no overt signs of toxicity (38). A single 100 mg/kg ip injection increased rat liver weight, total hepatic cytochrome P450, and cytochrome b_5 levels. The hepatic microsomal enzyme activity remained elevated 10

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Table 2 Pathologic changes induced by PCBs

Treatment	Animal	Liver	Kidney	Pericardium & Pericardium	Other Observable Changes	References
Single oral dose of 69 mg (47% Cl)	Guinea Pig Rat Rabbit	Small droplets through lobules, slight to moderate central atrophy, focal necrosis noted in a few animals.	Essentially normal	No noteworthy changes	Adrenals, spleen & pancreas showed no noteworthy changes	Miller [illegible]
300 mg daily for 6 days (64% Cl)	Rat	Cells swollen, hyaline granules present. Most died within few days.				Bennett [illegible]
50 mg daily for up to 6 months (61% Cl)	Rat	Enlarged (33% weight increase), large number of hyaline globules in cytoplasm. Several died during experiment.				Bennett [illegible]
33, 50 & 100 ppm in diet for 15 days (21-68% Cl Aroclors)	Rat	Increase in weight, effect increasing with increasing chlorine content. Aroclor 1233-10%, 1252-12%, 1254-14%, 1268-24% at 50 ppm				Street [illegible]
100 ppm in diet 200 ppm in diet 400 ppm in diet 800 ppm in diet (Aroclor 1242)	Chicken	No effect No effect Enlarged & Mottled Damaged	Damaged	Slight Hydropericardium Hydropericardium Hydropericardium Hydroperitoneum Enlarged		McCune [illegible]
200 & 400 ppm in diet for 2 weeks (42%)	Chicken	No changes noted	Paleness at 200 ppm, extensive hemorrhage, and enlargement at 400 ppm	Increased fluid in pericardial sac at the higher concentration	Paleness of pancreas, enlargement of adrenal and small spleen at low concentrations. At higher concentrations, pale creamy-colored pancreas, adrenals hemorrhage	Fisher [illegible]
Various doses (54% Cl, Aroclor)	Rengalese Finch	No weight changes	Weight was 32.4% of brain weight for contrast and 53.5% for those dying from PCB poisoning.	Slight weight increase, a few showed liquid in pericardial sac		Prestrett [illegible]
400 ppm in diet for 6-1 days (60% Cl[a])	Chicken	Centrolobular necrosis (compd. 1 & 2). Liver weight increased from 2.56 g/100 g to 4.31 g/100 g (compd. 3). Fatty degeneration.	Tubular dilation (compd. 1 & 2). Rate with compd. 3.	Hydropericardium common with compds. 1 & 2. Rare with compd. 3.	Increased porphyria, spleen small with reduction of red pulp and atrophy of white pulp (compd. 1 & 2). Spleen decreased from [illegible] (compd. 3)	Van [illegible]

[a]Phenoclor DP 6 (compd. 1), Clophen A60 (compd. 2), and Aroclor 1260 (compd. 3) were used. Differential [illegible] under numbers. All chickens died on compd. 1 and 2 within 60 days, only 15% mortality on compd. 3.

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days after a single dose of Aroclor 1242, suggesting that PCBs may be important in altering biological responses of mammals subjected to environmental chemical stress.

Kolter & Zinkl (59) compared the clinical and anatomical pathological effects produced by administering Aroclors 1221, 1242, and 1254 orally to rabbits once a week for 11 weeks. The livers in the 1254- and 1242-treated rabbits were significantly enlarged compared to the 1221-treated and control animals. The earliest change was megalohepatocytosis, followed by subcapsular midzonal necrosis. Fibrous connective tissue replaced the necrotic part of the lobules in the more severely affected livers. The rough endoplasmic reticulum in the livers of the 1254-treated rabbits appeared to have been destroyed, and there was also atrophy of the ulcers in the 1254 treated rabbits.

Dermal toxicity studies in rabbits of technical PCB samples that contain an average of 60% chlorine (Phenoclor DP6, Clophen A60, and Aroclor 1260) as well as fractions containing tetra and pentachlorodibenzofuran have been recently described by Vos & Beems (60). PCB-induced skin lesions were hyperplasia and hyperkeratosis of the epidermal and follicular epithelium following application of 118 mg of the three PCBs (5 times per week for 38 days) in the back skin of adult female New Zealand rabbits. Histopathology of the liver included centrolobular degeneration, centrolobular liver cell atrophy, focal necrosis, and cytoplasmic hyalin degeneration. PCB-induced kidney lesions were hydropic degeneration of the convoluted tubules and tubular dilation with the presence of casts. Definitive hyperplasia and hyperkeratosis of the follicular epithelium of the ear skin were seen after the topical application of fractions of Phenoclor and Clophen (eluted from chromatographic columns with 25% diethyl ether in hexane), while the fraction from Aroclor caused a minimal hyperplasia and hyperkeratosis of the follicular epithelium. Other effects elicited by the dermal application of the PCBs included thymus atrophy and lymphopenia as well as elevated excretion of fecal coproporphyria and protoporphyria.

From the response of the back skin and the liver of the rabbit to the three PCB mixtures, and from the response of the ear to the 25% diethyl ether-hexane fractions it was concluded that there were definite quantitative differences in toxicity, at least between the samples used in the above study (60) and prior studies (48). The extent to which these samples are representative of the normal commercial output has not been established and emphasizes the difficulty in the evaluation of toxicity data of PCBs in which the samples may differ in the amount and nature of toxic impurities.

Vos & Beems (60) also raised the possibility that since PCB is a porphyrogenic chemical, the skin lesions in man due to PCB may be caused by a combination of chloracne and acquired porphyria cutanea tarda.

Vos & Notenbrom-Ram (61) compared the toxicity of Aroclor 1260 with a single isomer 2,4,5,2',4',5'-hexachlorobiphenyl in New Zealand rabbits. Dermal applications of a total 120 mg Aroclor 1260 (5 times/wk for 28 days) resulted in early macroscopic skin lesions. The lesions in a 2,4,5,2',4',5'-hexachlorobiphenyl group of rabbits treated similarly appeared later and were less severe. Hyperplasia and hyperkeratosis of the follicular and epidermal epithelium were more severe in the Aroclor

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group. Enhanced liver weights were found in both test groups. Liver injury, as judged by light microscopic lesions and elevated serum transaminase levels, was somewhat more severe in the hexachlorobiphenyl group. Light microscopic findings included subcapsular necrosis, zonal necrosis, hydroscopic degeneration as well as peripheral and perinuclear shift of cell organelles, and focal cytoplasmic hyalin degeneration.

In electron microscopy the shift was due to a proliferation of smooth surfaced membranes of the endoplasmic reticulum (SER) resulting in a displacement of rough surfaced membranes (RER).

From the observed acnelike lesions, both from the PCB mixture and 2,4,5,2',4',5'-hexachlorobiphenyl, and assuming that the hexachlorobiphenyl is free from contamination with chlorinated dibenzofuran, we can conclude that this particular compound of the mixture PCB has a slight acnegenic action of itself. The major acnegenic action of crude PCB mixtures comes from chlorinated dibenzofurans and hepatic prophyria comes only from PCB itself.

It is important also to stress the toxic nature of the polychlorodibenzofurans. For example, tri- and tetrachlorodibenzofuran in a single oral dose of 0.5–1.0 mg/kg caused severe and often lethal liver necrosis in rabbits (62). The related compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin caused a lethal liver necrosis in the rabbit after a single oral dose of 0.05 mg/kg, and when applied to the ear again in a dose 10 times lower than that found to be effective in the case of chlorinated dibenzofuran, resulted in chloracne. Vos & co-workers (48) calculated a maximum dose/egg of 0.2 μg pentachlorodibenzofuran (obtained from Clophen A60), that caused 100% embryonic mortality when injected into the air cell of chicken eggs. The analogous effect was obtained with 0.03 μg hexachlorodibenzo-*p*-dioxin (63). The relationship between the toxic nature of PCB and the chick edema factor 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin has been described by Flick and co-workers (54).

b. Chronic Toxicity

The effects of low-level feeding (1,10, and 100 ppm) of Aroclors 1242, 1254, and 1260 to rats and dogs have been reported by Kehoe et al (64, 65). The only effect noted in dogs after 12 months of feeding was with Aroclor 1260 in a reduced rate of weight gain, increased liver weights, and elevated serum alkaline phosphatase in males at 100 ppm, and a reduced rate of weight gain in females at both 10 and 100 ppm. The effects seen in rats after 15 months of feeding were elevated liver and kidney weights (noted only with Aroclor 1260). However, the feeding of all three Aroclors led to an increase of liver weights and liver-weight-to-body-weight ratios at 24 months. Fatty degeneration, focal hyperplasia, and focal hypertrophy were also observed in some of the livers (64).

Kimbrough et al (66, 67) described morphological changes in livers of male and female Sherman strain rats fed 20, 100, 500, and 1000 ppm of Aroclors 1260 and 1254 in their diet for 8 months. Light microscopic changes consisted of hypertrophy of the liver cells, inclusions in the cytoplasm, brown pigment in Kupffer cells, lipid accumulation and, at the higher dietary levels, adenofibrosis. Ultrastructural changes of the livers of exposed animals consisted of an increase in smooth endoplas-

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rate reticulum and a typical mitochondria. Lipid vacuoles were occasionally surrounded by concentric membranes. The epithelial component of adenofibrosis consisted of goblet cells and cells that resembled the epithelium that lines the bile ducts. In general, the effect of Aroclor 1254 on the liver was more pronounced than that of Aroclor 1260.

Adenofibrosis was found in: (*a*) 1/10 males and 6/10 females at the dosage level of 100 parts per million Aroclor 1254 in the diet for about 8 months; (*b*) in 1/10 female rats at 100 parts per million Aroclor 1260 in the diet for about 8 months; and (*c*) in several male rats at 1000 parts per million Aroclor 1260 in the diet for 8 months (none at 500 parts per million).

The findings of Kimbrough et al in the above studies (66, 67), suggesting that the mammalian toxicity decreases as the level of chlorination increases, are in agreement with the conclusions of Lichtenstein et al (68) and Vos (26).

c. Reproductive Effects

A definite effect on reproduction in rats was produced by feeding 100 ppm of Aroclor 1254 (67). The first breeding was performed after 76 days on the treated diets and resulted in fewer offspring. The offspring at weaning were smaller and the survival was decreased compared to control animals. An increase in the liver weight in the F_{2a} generation of the weanlings at a dietary level of 20 ppm Aroclor 1254 was also found. A dosage of Aroclor 1260 equivalent to 100 mg/kg/day during the 7th and 15th days of pregnancy reduced the survival and number of young.

In another reproduction study in rats reported by FDA (29), Aroclors 1242, 1254, and 1260 were administered at levels of 1, 10, and 100 ppm in the diet. While Aroclor 1242 had no effect on the first generation, mating indices were low in the second generation at 100 ppm. The number of pups delivered and the number surviving to weaning were reduced in both the second and third litters with Aroclor 1254. Aroclor 1260 at a level of 100 ppm increased the number of stillborn animals. No effect was observed at levels of 1 and 10 ppm.

Keplinger et al (64) reported low mating indices and decreased survival of pups for animals receiving Aroclor 1242 at 100 ppm, and decreased survival of pups receiving Aroclor 1254 at 100 ppm. No reproductive effects were found with Aroclor 1260 at 1, 10, or 100 ppm, or with Aroclor 1242 or 1254 at 1 or 10 ppm (65). These studies suggest that in mammals reproductive effects decrease with increasing chlorination.

Keplinger et al (65), also reported that chickens fed 10 or 100 ppm of Aroclor 1242 or 100 ppm of 1254 exhibited loss of body weight, decreased thickness of egg shells, and poor hatchability of eggs. However, at 1, 10, and 100 ppm of Aroclor there were no adverse effects. While decreased hatchability was observed at 8 ppm of Aroclor 1242, there were no observed effects at 6, 4, or 2 ppm (64).

Studies reported by FDA (29) indicated that decreased egg production by chickens occurred at 100 ppm with Aroclors 1242 and 1254, but not with Aroclor 1260. Levels of 10 and 100 ppm of Aroclor 1242, and 100 ppm of Aroclor 1254 resulted in decreased eggshell thickness, which was not observed with Aroclor 1260 even at

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100 ppm. The hatchability of eggs was lowered at 10 and 100 ppm of Aroclor 1242 but not at 100 ppm of Aroclor 1254 or Aroclor 1260.

Scott et al (69) found decreased egg production at dietary levels of 10 and 20 ppm of Aroclor 1254 (10–13% reduction after 8 weeks). Hatchability of eggs was reduced at 10 and 20 ppm (up to 50% and 2.4%, respectively, after 8 weeks).

With a PCB level in the eggs of 2.2 ppm, hatchability of 3 and 4.5 ppm in eggs, the hatchability was reduced to about 56% of normal and almost to zero, respectively.

d. Carcinogenic, Teratogenic, and Mutagenic Effects

Kimbrough (67) observed bladder cancers in two rats fed 100 ppm of Aroclor 1260 over an 8-month period. Nagasaki et al (70) reported the hepatocarcinogenicity of Kanechlor-500 in male dd mice fed 500 ppm of PCB. The hepatomas appeared similar to those induced by δ-isomer of benzene hexachloride (71, 72), whereas Kanechlor-400 and Kanechlor-300 had no carcinogenicity activity in the liver of mice.

Kimura & Baba (72) described neoplastic changes in the rat liver induced by Kanechlor-400. The PCB administered to rats in the diet at 38.5–462 ppm induced a benign neoplastic change in the liver, which appeared exclusively in the female. All the rats that ultimately ingested > 700 mg of Kanechlor-400 showed hypertrophy of the liver, while pinhead to pear-sized round and pale brown flecks or nodules were scattered on the surface and on the cut surface of the liver of all female rats ingesting > 1200 mg, but on none of the male rat livers. Fatty degeneration and multiple adenomatous nodules in the liver, lung abscesses, pneumonia, splenatrophy, and intracranial abscesses were found frequently in experimental animals of both sexes, and depilation was observed in females ingesting > 600 mg of Kanechlor-400.

Allen & Norback (73) reported the induction of hyperplasia and dysplasia of the gastric mucosa in subhuman primates (male rhesus monkeys ranging in age from 1.5–2 years and having an average weight of 2.9 kg) fed a diet containing 300 ppm of Aroclor 1248 or 5000 ppm of polychlorinated triphenyl (Aroclor 5460) (PCT) for 3 months. During the course of the experiment, the animals were given access to 400 g of the experimental diet daily. Within 1 month, all of the PCB fed animals and within 6 weeks, all of the PCT fed animals (6) had hair loss from the head, neck, and back. A progressive, generalized, subcutaneous edema, particularly of the face, was manifested as swollen eyelids and lips.

The concentration of PCB within the experimental diet was less than an order of magnitude greater than that occurring in random food samples sold in the United States and less than levels that have occurred in food products as a result of industrial accidents. The increased cellularity, abnormal dysplastic growth pattern, and invasion of the adjacent tissue region indicate compromised gastric function and were believed by the authors to be *suggestive* of an eventual neoplastic transformation. However, the carcinogenic potential could not be evaluated from a short-term study.

McLaughlin et al (74) reported that Aroclor 1242 gave no hatch at a level of 25 mg when injected into chicken yolk sac. At a level of 10 mg/egg embryos were found

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with leg deformities, edema, and growth retardation. Carlson & Daby (75) found that injection of Aroclor 1242 into chicken eggs on day 0 of incubation severely limited hatchability at levels above 2.5 ppm. The 1254 and 1260 isomers were less effective in this respect, requiring 10 ppm or more. Embryonic mortality occurred during the period of organ formation, suggesting an effect on inductive mechanisms (several malformations in chicks that developed until day 21). The effects induced by the day 0 injection of the 1242 isomer were permanent, as growth rates were severely depressed during the 2 week period following hatching.

Aroclor 1254 was found to be fetotoxic to the rabbit at amounts of 12.5 mg/kg and above as evidenced by abortions, maternal deaths, and stillborns (76). The fetotoxic effect did not appear to be dose related nor was it influenced by the period of administration. The rat did not appear to be as sensitive a species, as doses up to 100 mg/kg did not cause fetal deaths or malformations. Dead fetuses from treated animals showed no consistent skeletal abnormalities. Oral administration of 12.5–50 mg/kg/day did induce abortions and was fetopathic to rabbits when treated for the first 28 days of gestation. In a preliminary study (76) the oral administration of 50 mg/kg of Aroclor 1254 to 6 pregnant rabbits 5 days a week during their gestation period also caused abortions and fetal deaths.

Peakall et al (77) reported that embryos from the second generation of Ring doves (*Streptopelia risoria*) fed 10 ppm Aroclor 1254 exhibited a high frequency of chromosomal aberrations and a high incidence of embryonic death.

No chromosomal aberrations were observed in human lymphocyte cultures exposed to Aroclor 1254 at 100 ppm (78), and Keplinger et al (64), employing a dominant lethal assay, reported no evidence of mutagenic effects of Aroclors.

Green et al (79) described the cytogenetic effects of Aroclor 1242 on rat bone marrow and spermatogonial cells. Aroclor 1242 was given to albino Osborne-Mendel rats as an acute dosage (PO) at 5000, 2500, and 1250 mg/kg or as a subacute regimen at 500 mg/kg and as a solution in corn oil at the other levels.

The results from the bone marrow study showed no significant increases in chromosomal abnormalities or inhibition of cellular division. The study of spermatogonial cells showed significant increase in abnormalities but statistically significant decreases in the number of dividing spermatogonial cells ($P < 0.05$). This effect was noted at 500 X 5 and 5000 X 1 dosages. It was concluded that Aroclor 1242 does not produce chromosomal abnormalities in rat bone marrow or spermatogonia but does cause, at relatively high dosages, a decrease in the number of dividing spermatogonial cells.

c. *Immunosuppressive Effects*

An interaction of PCBs with duck hepatitis virus was found by Friend & Trainer (80). Ten-day-old ducklings fed Aroclor 1254 at 25, 50, and 100 ppm of PCBs for 10 days suffered no apparent clinical intoxication but when challenged with duck hepatitis virus 5 days later suffered higher mortality than ducklings not exposed to PCB but challenged with the virus.

The effect of PCB (Clophen A-60 and Aroclor 1260) feeding at levels of 0, 10, 50, and 250 ppm on the humoral and cell-mediated immune response was described by Vos & Van Dinel-Grootenhuis (81). A suppression to the humoral immunity was

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found at the 50 ppm level in guinea pigs, after stimulation with one dose of tetanus toxoid (alum-adsorbed). The number of tetanus antitoxin-producing cells in the stimulated popliteal lymph nodes was reduced. Stress was not considered responsible for the reduced immunological responses. A high mortality occurred at the 250 ppm level. Cachexia and depletion of the lymphoid system and liver damage were the most important findings in these animals.

The immunosuppressive activity of Aroclor 1260 (10 and 50 ppm over 8 weeks) on the humoral immune response in female albino guinea pigs was studied by Vos & Beroij (82), using tetanus toxoid as a function test of the immunological system in half the animals. Cellulose acetate electrophoresis was used to determine the seven proteins, including the γ-globulin level. The number of γ-globulin containing cells in the popliteal lymph nodes was determined semiquantatively with the direct fluorescent antibody technique. (Both techniques were sensitive parameters for the immunosuppressive action of PCB in the tetanus toxoid stimulated animals.)

Vos & Deems (60) found a reduced number of white blood cells, atrophy of the cortex of the thymus, and a reduction in the number of germinal centers in the spleen and lymph nodes after dermal application of high doses of PCBs in rabbits. This suggests the possibility of an immunosuppressive action.

Feeding guinea pigs 10 ppm Aroclor 1260 for 8 weeks (26) resulted in a decreased number of antibody-forming cells in the popliteal lymph nodes, after stimulation of the humoral lymphoid system with tetanus toxoid. Vos (26) suggested that this suppression may explain the higher sensitivity of PCB-fed ducklings for duck hepatitis virus (80).

Small spleens were noted in chickens fed PCBs, showing atrophy of the lymphoid system (52, 53).

f. Human Effects

In 1968, an outbreak of poisoning that involved at least 1000 people occurred in Northern and Western Japan where rice bran oil was contaminated with Kanechlor-400. This PCB contains 48% chlorine and has as its main components 2,4,3',4'-, 2,5,3',4'-, 2,3,5,4'- and 3,4,3',4'-tetrachlorobiphenyl, and 2,3,5,3',4'-pentachlorobiphenyl (83). The disease was named "Kanemi Yusho" (34, 84). The contamination occurred because PCBs used as heat exchangers in the manufacturing process leaked into the oil through pin holes in the pipes. Umeda claimed (33) that there are an estimated 15,000 victims of Kanemi Yusho although only 1081 persons have been officially diagnosed as such.

Exposure levels to the oil were calculated to approximate 15,000 mg/day. The lowest reported figures allow an estimate of a minimal positive effect level at 3 mg PCB per day over several months. However, the average doses associated with significant disease in the "Yusho" incident were much higher and were in the range of 30 mg/day (15, 28). The latency period between ingestion of the oil and the onset of clinical signs and symptoms was estimated at 5–6 months (3).

The clinical aspects associated with Yesho included: chloracne, blindness, systemic gastrointestinal symptoms with jaundice, edema, and abdominal pain. Chloracne is very persistent, with some patients showing evidence of it after 3 years. Table

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Table 3 Subjective symptoms complained of by Yusho patients[a,b]

Symptom	Male (%)	Female (%)
Dark brown pigmentation of nails	83.1	75.0
Distraction of hair follicles	64.0	56.0
Increased sweating at palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red plaques on limbs	20.2	16.0
Itching	42.7	52.0
Pigmentation of skin	75.3	72.0
Swelling of limbs	20.2	41.0
Stiffened sole and palm	24.7	29.0
Pigmented mucous membrane	56.2	47.0
Increased eye discharge	88.8	83.0
Hyperaemia of conjunctiva	70.8	71.0
Transient visual disturbance	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Feeling of weakness	58.4	52.0
Numbness in limbs	32.6	39.0
Fever	16.9	19.0
Hearing difficulties	18.0	19.0
Spasm of limbs	7.9	8.0
Headache	30.1	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

[a] Eighty-nine male and 100 female patients diagnosed before October 31, 1968 were examined.

[b] From a report of "Yusho, A Poisoning Caused by Rice Oil Contaminated with Chlorobiphenyls" (Ref. 84).

3 lists the subjective symptoms of 89 male and 100 female Yusho patients. The severity of the disease varied with age, being greatest from adolescence through 40 years (85). The disorder generally cleared when exposure to the offending agent was discontinued.

Newborn infants of poisoned mothers had skin discoloration due to the presence of PCB via placental passage. (The dark skin discoloration regressed after a period of 2–5 months). Gingival hyperplasia with pigmentation was seen in several cases. Decreased birth weights were also noted, but no evidence could be obtained in regard to the possible retardation in physical and mental activities of the babies (86). The skin of stillborn infants showed hyperkeratosis and atrophy of the epidermis, and cystic dilation of the hair follicles. Residues of PCB have been found in fetal tissue (87, 88).

Examination of autopsy tissues of two Yusho fatalities revealed the presence of chlorobiphenyls in all of the examined organs, especially mesenterial fatty tissues, skin, and bone marrow (37, 89). PCBs were found with longer retention times

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(probably pentachlor- and higher chlorinated biphenyls) in autopsy tissues, and it was assumed that their presence might have been responsible for the observed long duration of the intoxication symptoms.

Additional detailed clinical and experimental studies have been made regarding the clinical features of Yusho and concerning toxicological effects on laboratory animals (34–37). Among the miscellaneous observations of biochemical and physiological abnormalities in Yusho patients are: increased urinary 17-ketosteroid excretion, respiratory distress with secondary infection of the upper respiratory tract, a hematological picture suggestive of acute or chronic inflammation, and elevated blood-serum triglycerides.

Chloracnegen effects were reported as early as 1936, following industrial exposure to the PCBs (32, 90–96). Approximately 10 cases of fatal intoxication involving persons who handled or were exposed to chlorinated biphenyls or naphthalenes in their occupations have been described (94, 96). In all cases histological examination revealed liver fatty degeneration necrosis and cirrhosis.

In contrast to the reports of industrial PCB poisoning, the outstanding difference in Yusho disease is the frequent occurrence of hyperpigmentation of the skin, as seen in 72% of women and 75% of men, which may be due to the differences in route of intake. The Yusho cases resulted from oral intake while industrial cases resulted from dermal exposure. Liver damage (in contrast to occupational poisoning) was not marked in the Yusho cases.

Two surveys of human adipose tissue in the USA (44, 45) gave broadly similar results with means of the order 1.0 ppm PCB. Biros et al (43), however, have reported 200 and 600 ppm of PCB in samples of human adipose tissue, and Price (44) reported that one autopsy case contained 115–240 ppm in fat.

Mean levels of PCB in human blood plasma were reported by Finklea et al (38) to be in the order of 2.0 ppb. In this study, rural Negroes had much lower levels in blood plasma (0.35 ppb) than the overall. Rural whites had slightly higher levels (near 3.2 ppb). Interestingly, this pattern was opposite to that of Σ DDT, in which rural Negroes had by far the highest levels (38).

Risebrough & Brodine (47) reported the mean PCB level in human milk from two cities in California to be about 60 ppb. Mean levels in human milk in Sweden and Germany were 16 ppb and 100 ppb respectively (41, 46). It has been calculated that, based on a daily milk intake of 150g/kg, breast-fed infants in California ingest some 9/μg/kg/day of PCBs. The toxicological consequences of body burdens of PCB are difficult to assess at this time.

g. Miscellaneous Toxicological and Biological Effects

Aroclor 1254 was found to potentiate the toxicity of carbon tetrachloride in a manner similar to that reported for DDT (28). The studies of Grant et al (97) suggest that the liver is the main site of Aroclor 1254 metabolism, because rats with carbon tetrachloride damaged livers were unable to metabolize this mixture of chlorinated biphenyls as rapidly as rats with normal livers. Aroclor 1254 significantly increased the size of the liver and also the percentage of lipid in the liver. The same study revealed that the components of Aroclor 1254 with the shorter GLC retention times,

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presumably with the lowest chlorine content (PSI) were metabolized to a greater degree than those with the longer retention times. This effect is in agreement with the studies of Phenobarbital DDE fed to Japanese quail (99).

Street and co-workers (25) studied the effects of diets of 50 ppm to 100 ppm of 10 Aroclors ranging in chlorine content, and 21%–68% fed to rats for 15 days. Their effect on sleeping time induced by hexobarbital, in vitro rates of aniline hydroxylation, and demethylation of *p*-nitroanisole, and the rate of excretion were all found to be increased with increasing chlorine content. Aroclor 1221 (50 ppm) reduced hexobarbital sleeping time by 11%, whereas for Aroclor 1248 and 1268 the figures were 35% and 48%, respectively. Liver weights also increased with increasing chlorine content of the Aroclors. The storage of dieldrin was decreased in relationship to the chlorine content. For example, with Aroclors containing 60% chlorine or more, the storage in adipose tissue was reduced to the levels found in untreated control animals. The induction of PCBs of hepatic microsomal hydroxylating enzymes has been demonstrated in the American kestrel (Peakall & Lincer 7) and pigeons (Risebrough et al 5).

Villeneuve et al (101) studied the effects of PCB administration on microsomal enzyme activity in pregnant rabbits. The nil-effect level of Aroclor 1254 for enzyme induction in the pregnant rabbit is between 1.0 and 10 mg/kg body weight when administered for 28 days during gestation. Aroclor 1221 induced no enzyme activity in the does, fetus, or placenta, so its nil-effect level must be considered higher than that for Aroclor 1254. Placental transfer was shown to occur for both Aroclor 1254 and 1221 but causes no changes in the biochemical physiological parameters measured, e.g. total amount of Vitamin A stored per liver, protein levels, aniline hydroxylase enzyme activity, serum cholesterol, no effect in reproductive processes. The drug-metabolizing enzymes aniline hydroxylase and aminopyrine-*n*-demethylase were both induced by 10 mg/kg Aroclor 1254.

Litterst & Van Loon (100) studied enzyme induction by equimolar dietary amounts of DDT, phenobarbital, and Aroclor 1254 after 30 days of treatment. At 150 μ mole/kg of food, the PCB was far more effective than phenobarbital, and at least as effective as DDT. At 15 μ mole, phenobarbital, DDT, and Aroclor 1254 produced substrate-specific increases in enzymatic activity.

Ito and co-workers (102) found that the administration of PCBs to rabbits increases the total lipid, triglyceride, and cholesterol content of liver and decreases the total liver phospholipid content. (The concentration of serum triglycerides was abnormally increased.)

Lincer & Peakall (24) demonstrated an inductive effect on estradiol metabolism from the administration of Aroclor 1254 and Aroclor 1262 to American kestrels. Aroclors 1221, 1232, 1242, and 1248 also have an estrogenic effect on the rat uterus, which was not shown with Aroclors of higher chlorination (22). The estrogenic activity was evaluated using the 18-hr glycogen response to the immature rat after a single subcutaneous injection.

Phitonow & Funnell (23) reported an antiandrogenic-like effect in cockerels when Aroclor 1254 was incorporated in the diet at 250 ppm for as little as 6 weeks. Vos (26) suggested that both increased steroid metabolism as cited by Rehfeld et al (51)

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and the estrogenic activity could be responsible for the depression of secondary sexual characteristics such as decreased development of comb and wattles noted in cockerels (23).

Örberg et al (21) described the prolongation of estrus cycle in NMRI-strain mice given single ip administrations of DDT (40 mg/kg) and Clophen A-60 (20 mg/kg). The prolongation appeared to decrease with time, the lengths returning to normal after 3 cycles. The effects observed probably indicated that the chlorinated hydrocarbons affected the catabolism of steroid hormones. No changes were found in the frequences of cornified cells after the ingestions. A prolonged estrus cycle implies less frequent periods of sexual receptivity in the female and hence could cause a decline in the reproductive capacity of the animals.

Ogawa (103) demonstrated neuropathy in rats following administration of PCB (0.3–0.5 ml/kg/day) for 14 or 21 days as evidenced by marked or moderately impaired motor function, decreased motor conduction velocity, and loss of large nerve fibers.

Norback & Allen (104, 105) reported that ingestion of PCBs by rats for 1–5 weeks resulted in liver hypertrophy, with proliferation of the smooth surfaced membranes of the endoplasmic reticulum (SER) and formulation of large concentric membrane arrays within the cytoplasm of the hepatic cells. These concentric membrane arrays were suggested by Vos (26) as probably representing the hyalin bodies described by Bennett et al (106) and Miller (49) and could have an enzymatic function similar to that associated with the SER (104, 105).

Yap et al (107) reported that PCBs inhibit the activity of fish ATPases in vitro. Both Mg^{2+}ATPase and NA^{+}-K^{+}-ATPase were inhibited in brain, kidney, and liver at concentrations as low as 0.03 ppm. An analogous effect for DDT has been cited in the above study.

h. Summary and Conclusions

Despite the increasing number of mammalian toxicological investigations, aspects of *definitive* acute, subacute, and chronic toxicity of the polychlorinated biphenyls still remain poorly known as regards man. The chemical and physical properties, e.g. the stability, complexity, and heterogeneity of the commercial formulations per se, the difficulty of separation and analysis as well as the non- or ill-defined nature of the material actually used or reported in many studies, conspire in making the evaluation of toxicity and biological data difficult.

A number of the more salient biological and toxicological aspects of the PCBs are summarized in Table 4. It is possible to distinguish two actions of the PCBs on mammals, e.g. liver damage and skin lesions. Liver damage has been predominantly manifest in mice, rats, guinea pigs, rabbits, and monkeys in feeding and to a lesser extent in inhalation studies. Liver damage and transplacental transmission as shown in abnormal pigmentation and miscarriages have also been observed in the Yusho poisonings in Japan. Skin lesions have been observed in rabbits in dermal toxicity studies. In addition, chloracnegenic and porphyrogenic as well as edema effects in many species have been caused by commerical PCB preparations. The liver damage and skin lesions are believed to be caused primarily by chlorinated dibenzofuran contaminants and to a minor extent by PCB itself. These contaminants are also

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responsible for the edema formation observed in fowl, while the PCBs are suggested to the causative agent for the hepatic prophyria.

In contrast to the acute toxicity, aspects of subacute and chronic toxicity appear to be of far greater concern. In rats the acute LD_{50} is of the order of 5–10 g/kg. Chronic intake of relatively small doses of PCBs, however, has been demonstrated to have adverse effects in man and other vertebrates. For example, in man it has been estimated that as little as 10 mg/kg over 50 days causes chloracne. It has also been shown that toxic effects during continuous exposure at low levels may only appear after extremely prolonged intake, e.g. certain reproductive effects of Aroclors 1242 and 1254 in rats were not manifest until after 15 months of prolonged intake.

Although deaths from exposure, either acute or chronic, have not been clearly documented for man, aspects of the toxicity as described above coupled with increasing reports of the ubiquity of the presence of PCBs in humans (analogous to DDT and DDE) suggest further study is essential to elaborate more definitely the potential toxicity of this environmental pollutant. In this regard further toxicologi-

Table 4 Some toxicological and biological effects of the PCBs

1. Acute oral LD_{50} in mammals varies from approximately 2–10 g/kg. (Apparent increase in mammalian toxicity with decrease in chlorine content.)
2. Induction of hyperplasia and dysplasia of gastric mucosa in subhuman primates.
3. Enlargement of the liver and vacuolar or fatty degeneration of liver cells in rats, guinea pigs, and monkeys.
4. Hepatocarcinogenicity in mice and bladder carcinogenicity in rats.
5. Production of hydropericardial edema in chickens and Japanese quail.
6. Teratogenic effect in chick embryo.
7. Ceratoxicity in rabbit.
8. Adverse reproductive effects in rats at levels of ca 100 ppm in diet.
9. Adverse reproductive effects in mink.
10. Enhanced chromosomal aberrations and embryonic death in ring doves.
11. Effects on hatchability in chickens, Japanese quail.
12. Skin, liver, and kidney lesions in rabbits following dermal exposure.
13. Immunosuppressive effects in rabbits.
14. Chemical porphyrogenic effects in many species.
15. Chloracnegenic and hepatotoxic effects in man.
16. Hyperplyceridemic effects in man.
17. Human miscarriages, stillbirths, and transplacental transmission in abnormal pigmentation from "rice-oil disease" (Yusho).
18. Hepatotoxic, chloracnegenic, and porphyrogenic effects of chlorinated dibenzofuran contaminants in several species.
19. Potentiating of toxicity (e.g. carbon tetrachloride) in rats.
20. Chloracnegenic effects of chlorinated naphthalene contaminants in man.
21. Generally, enzyme induction increases with increase in chlorination of PCBs.
22. Induction of hepatic hydroxylating microsomal enzymes and increased estrogenic activity in the rat.
23. Inductive effect on estradiol metabolism and anti-androgenic effects in kestrels.
24. Prolongation of estrus cycle in mice.
25. Inhibition of ATPases in vitro.

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cal and biological studies involving key individual characterized PCB isomers, [illegible] contaminant chlorinated dibenzofuran and naphthalenes, definitive pharmacogenetic elaborations (perhaps in primates), and more definitive mutagenic and teratologic studies should all help in this most needed assessment.

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