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Reproductive Toxicity of Commercial PCB Mixtures: LOAELs and NOAELs from Animal Studies

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This paper reviews the developmental/reproductive toxicity of commercial polychlorinated biphenyl (PCB) mixtures in animals and reports on the "no-observable-adverse-effect levels" (NOAELs) and "lowest-observable-adverse-effect levels" (LOAELs) from these studies. Identification of the lowest effective doses for reproductive toxicity of PCB mixtures is difficult because a variety of reproductive and developmental effects have been reported in several species using different commercial mixtures. Factors to be considered include sensitivity of the end point, sensitivity of species, study quality, biological plausibility, and relevance to humans. End points affected at the lowest doses (sensitive end points) included postnatal growth, development, and function. Among species for whom sensitive end points have been evaluated, a LOAEL of 0.25 mg/kg/day was identified for rodents on the basis of developmental delays in growth and behavioral function, and a LOAEL of 0.008 mg/kg/day was identified for nonhuman primates based on postnatal skin hyperpigmentation. NOAELs were not identifiable for these sensitive end points because effects were reported at the lowest doses tested.

Introduction

Polychlorinated biphenyls (PCBs) are a group of biphenyl ring chemicals that contain one to nine chlorine atoms per molecule. Commercial PCB mixtures are identified by their percent chlorine content (on a weight basis). Commercial PCB mixtures have had wide use as coolants and lubricants. Although production was banned in 1979, PCBs continue to be a health concern because they persist in the environment (*1*). PCBs are classified as a priority pollutant by the United States Environmental Protection Agency (EPA), and as one of the 100 most significant hazardous substances by the Centers for Disease Control (CDC) (*2*).

Commercial production of PCBs began in the U.S. in 1929. In retrospective studies of museum specimens of Lake Michigan fish, PCB residues first appeared in 1949, and levels increased progressively through 1965, the last year studied (*3*). In 1966, pesticide surveys in Sweden first reported high levels of PCB residues in fish and bird tissues (*4*). Subsequently, PCB residues were detected in a variety of birds, fish and marine mammals around the world, indicating pervasive contamination of environmental media and entry into the food chain.

PCB reproductive effects in animals first received extensive experimental examination in 1968 in connection with a reproductive failure syndrome seen in domestic mink (*5*). Investigators traced the cause of this syndrome to fish in the diet, then to fish originating in the Great Lakes, and finally to the PCB content of the fish. For this reason, the database on reproductive toxicity of PCBs is unusual in that it contains extensive information on effects in mink.

Adverse health effects in humans, including developmental and reproductive effects were first associated with PCB exposures in 1968 when the Yusho syndrome was identified in Japanese who consumed rice oil contaminated with commercial PCBs (*6*). Subsequently, health effects associated with human exposures to PCBs via the food chain have been studied in the U.S. (*7*). Developmental toxicity has been major focus of these studies (*8*).

In the past 20 years, PCB reproductive toxicity has been investigated in a large number of studies of humans, laboratory animals, and wildlife (*9–11*). This paper reviews studies of land mammals to select LOAELs (lowest-observable-adverse-effect levels) and NOAELs (no-observable-adverse-effect levels) for the reproductive and developmental effects of PCB mixtures from this large volume of data. The NOAEL (or LOAEL if a NOAEL cannot be identified) is used in the risk assessment process as the basis for calculation of a reference dose (RfD), an exposure level expected to pose minimal risk to humans (*12*). Regulatory exposure levels designed to protect human health are typically based on cancer risk (*13*) because PCBs are probable human carcinogens (*14*) and because cancer risk occurs at lower exposure levels than most other toxic effects. However, regulatory exposure levels for reproductive effects are sometimes required. For example, California's Proposition 65 legislation (*15*) requires warnings to be provided and discharges to drinking water to be prohibited for substances that are reproductive toxicants, as well as those that cause cancer.

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Table 1. Reproductive end points affected by PCBs (41–60% chlorination).[a]

End point	PCB	Species	Reference
Lower reproductive organ weights in exposed males	Aroclor 1254	Mice	(19)
	Clophen A60	Mice	(20)
Lower numbers of sperm in exposed males	Aroclor 1254	Mice	(19)
	Clophen A60	Mice	(21)
	Aroclor 1248	Rhesus monkeys	(22)[b]
Altered estrous/menstrual cycles	Clophen A60	Mice	(23)
	Clophen A60	Mice	(24)
	Aroclor 1254	Rats	(25)
	Aroclor 1248	Rhesus monkeys	(26)[b]
Lower number of exposed females mated	Aroclor 1242	Rats	(27)
	Aroclor 1254	Rats	(25)
	Aroclor 1242	Mink	(28)
	Aroclor 1248	Rhesus monkeys	(26)[b]
Lower maternal weight gain during pregnancy	Aroclor 1254	Rats	(29)
	Aroclor 1254	Rats	(30)
	Kanechlor 500	Rats	(31)
	Aroclor 1254	Rabbits	(32)
	Aroclor 1248	Rhesus monkeys	(26)[b]
Fewer completed pregnancies	Aroclor 1254	Rats	(29)
	Aroclor 1016	Mink	(28)
	Aroclor 1242	Mink	(28)
	Aroclor 1248	Rhesus monkeys	(26)[b]
Greater incidence of malformations	Kanechlor 500	Rats	(33)
	Aroclor 1254	Mice	(34)
Fewer offspring/litter	Aroclor 1254	Rats	(32)
	Aroclor 1254	Rabbits	(35)
	Aroclor 1254	Rats	(36)
	Aroclor 1260	Rats	(36)
	Aroclor 1016	Mink	(28)[b]
	Aroclor 1254	Rats	(37)
	Aroclor 1254	Rats	(25)
	Aroclor 1242	Swine	(38)
Lower offspring birth weights	Kanechlor 300	Rats	(39)
	Kanechlor 500	Rats	(31)
	Aroclor 1254	Rats	(30)
	Aroclor 1248	Rhesus monkeys	(40)[b]
	Aroclor 1016	Rhesus monkeys	(41)
	Aroclor 1254	Rats	(29)
Less postnatal survival of offspring	Aroclor 1254	Rats	(36)
	Aroclor 1260	Rats	(36)
	Kanechlor 500	Rats	(31)[b]
	Aroclor 1016	Mink	(28)[b]
	Aroclor 1254	Rats	(29)
	Aroclor 1254	Mice	(42,43)
	Aroclor 1248	Rhesus monkeys	(44)[b]
Lower postnatal weight gain in offspring	Aroclor 1254	Rats	(36)
	Aroclor 1016	Mink	(28)[b]
	Aroclor 1254	Mink	(45)
	Aroclor 1254	Rats	(25)
	Aroclor 1254	Rats	(29)
	Aroclor 1254	Mice	(42,43)
	Aroclor 1248	Rabbits	(46)
Lower reproductive organ weights in offspring	Aroclor 1254	Mice	(42,43)
	Aroclor 1254	Rats	(47)
Impaired function in offspring	Aroclor 1254	Rats	(37)
	Aroclor 1254	Rats	(29)
	Kanechlor 500	Rats	(31)
	Aroclor 1254	Rats	(47,48)
	Fenclor 42	Rats	(49)
	Aroclor 1254	Mice	(50)
	Aroclor 1248	Rhesus monkeys	(44,51)[b]
	Aroclor 1016	Rhesus monkeys	(52,53)
	Aroclor 1254	Mice	(42)

[a]Direction of effect relative to controls is stated along with agent, species, and reference. Only mammalian species are included.

[b]Effects occur at doses with substantial maternal toxicity.

Table 2. Chlorine content of some commercial PCB mixtures.

PCB	% Chlorine
Aroclor 1221	21
Clophen A30	30
Kanechlor 300	41
Aroclor 1016	41
Aroclor 1242	42
Fenclor 42	42
Aroclor 1248	48
Aroclor 1254	54
Kanechlor 500	54
Aroclor 1260	60
Clophen A60	60

NOAELs can be based on exposure levels from either human or animal studies. However, human PCB exposures are difficult to characterize. Human exposures typically occur via diet, and both the type and level of PCB contamination can vary widely with the source of dietary components. Analysis of human tissues and body fluids does not reveal the type and level of the exposure because biologic and physical transformations that occur in the environment alter the composition of PCB mixtures. Further, bioaccumulation of PCBs makes it difficult to identify time of exposure and peak exposure from tissue analysis. Confounding of exposures with other organochlorine toxicants such as DDT, polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzodioxins (PCDDs) is also a problem. Thus, NOAELs for RfD development are likely to come from animal studies, provided that human relevance can be established.

Table 1 outlines the reproductive/developmental toxicity end points that have been demonstrated to be affected by commercial PCB mixtures in animals. The chlorine contents of the various PCB mixtures referred to in this review are given in Table 2. This tabulation is limited to studies using PCB mixtures with 41 to 60% chlorination because most of the available studies have used these mixtures. The table does not include the few studies available for Aroclor 1221 (21% chlorine) (*51,52*) or the numerous studies using individual congeners present in PCB mixtures.

Most animal studies provide values that can be interpreted as NOAELS or LOAELS. Choice of a particular NOAEL or LOAEL for human health risk assessment, however, requires a number of considerations. The NOAEL or LOAEL should be identified for the most sensitive effect in the most sensitive species. Information on the shape of the dose-response curve and the severity of effects at the next higher dose level should be taken into account (*53*). The NOAEL or LOAEL should be derived from a study of good quality, and information on biological plausibility and relevance to humans should also be considered. These issues are discussed below.

Identification of NOAELS: Sensitive End Points

Initial evaluation for sensitive end points was made by reviewing studies on rats and mice, since information is available for most end points in these species. In Figure 1, LOAELS for reproductive and developmental toxicity end points are indicated to the right. To the left, LOAELs for other kinds of effects identified in the same group of studies are presented. To convert dietary PCB levels to equivalent milligram per kilogram per day doses for rats and mice, standard conversion factors for food

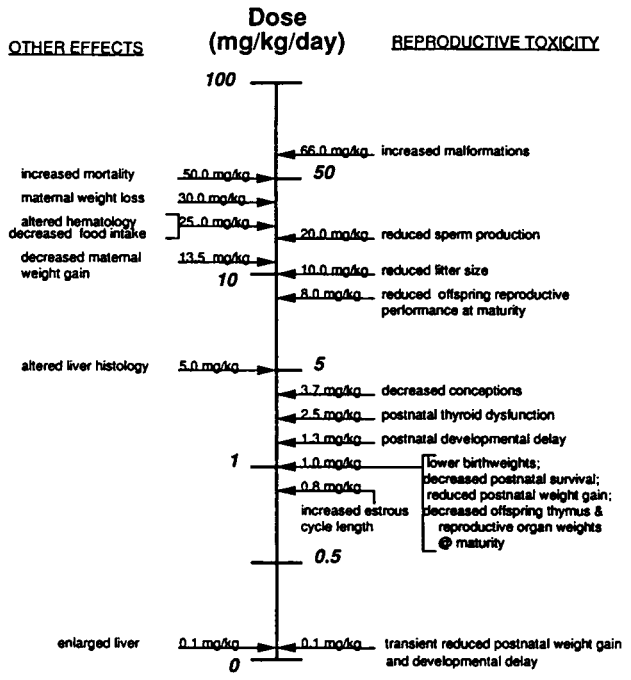

FIGURE 1. Relationship between dose and LOAELS for various reproductive/developmental toxicity end points (right) and other end points (left). Includes studies done in rats and mice with commercial PCB mixtures containing 41 to 60% chlorine. The majority of these studies were conducted with oral dosing via diet; conversion to milligram per kilogram per day doses was ppm × 0.05 for rats and ppm × 0.13 for mice (*61*).

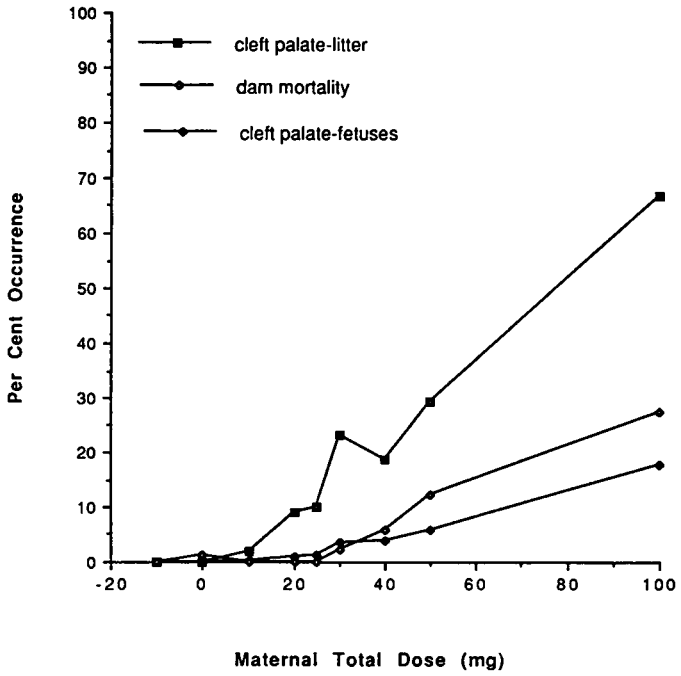

FIGURE 2. Dose-response curves for general toxicity and teratogenic actions of Kanechlor 500 in mice. Data from Watanabe and Sugahara (*33*).

consumption and body weight were used.

Figure 1 indicates a continuum of reproductive/developmental effects over a dose range of 0.1 to 66 mg/kg/day. Other adverse effects begin to appear at doses of 5 mg/kg/day, and PCBs have biological actions such as stimulation of hepatic metabolism and enlargement of liver (*54*) at lower doses. Reproductive/developmental effects seen at the higher doses (teratogenicity, male reproductive effects, embryo/fetal loss) are not likely to serve as the basis of a NOAEL. They are discussed briefly below. The more sensitive end points, primarily effects on ovulatory cycles and on postnatal growth and function, are discussed in more detail.

Male Reproductive Effects, Embryo/Fetal Loss, Teratogenic Effects

Indices of male reproductive function (decreased sperm count and smaller male reproductive organs) and embryo/fetal loss in dosed females (decreased litter size, increased resorptions) are seen at dose levels that produce other adverse effects (decreased food intake and weight gain). The relationship between general and reproductive toxicity at these dose levels is not clear; reproductive effects may be secondary to general toxicity, or they may simply occur in the same dose range.

Teratogenic effects have the highest LOAELs of any reproductive effect based on results from one study in mice (LOAEL 66 mg/kg/day) (*33*) and one study in rats (LOAEL 244 mg/kg/day) (*34*). While other studies (*31,39*) have failed to demonstrate teratogenicity in mice and rats after exposure during embryogenesis, doses were lower and group sizes smaller in these studies.

The production of teratogenic effects only by exposure to high doses suggests either that PCB mixtures are not potent teratogens or that their teratogenic effects are secondary to maternal toxicity. The latter explanation seems less likely since teratogenic effects appeared at dose levels that did not increase maternal mortality in one study that used maternally toxic doses (*33*) (Fig. 2). Also, while teratogenic doses would be highly toxic under chronic conditions, they do not approach acute lethal doses, and the exposure periods for the positive teratology studies were brief [1 day (*34*), 5 days (*33*)]. These considerations suggest that maternal toxicity is not required for the teratogenic effect. Demonstration of teratogenic effects in birds after either maternal (*55*) or *in ovo* (*56*) treatment is consistent this suggestion.

It is more likely that commercial PCB mixtures have low teratogenic potency. PCBs are thought to exert their teratogenic effects via the same mechanism as tetrachlorodibenzodioxin (TCDD), a widely recognized teratogen. Individual PCB isomers which are coplanar are structurally similar to TCDD and are teratogenic (*57*). These isomers are present low amounts in commercial mixtures, and this may account for the low teratogenic potency of mixtures. PCDF content may also be relevant to the teratogenic action of particular commercial mixtures.

Effects on Estrous Cycle and Conception

Four studies that specifically examined estrous cycle and conception are outlined in Table 3. Prolonged estrous cycles were found in mice fed 0.8 mg/kg/day Clophen A60 for a 10-week period (*23*) or given a single IP injection of 20 mg/kg (*24*) and also in rats given 10 mg/kg/day Aroclor 1254 for 6 weeks (*25*).

Table 3. Studies investigating PCB effects on female fertility.

Study	Agent	Dose/duration	Dosing	Unit of Analysis	Data
Brezner et al., 1984 (*25*)	Aroclor 1254	10 mg/kg/day, 6 weeks	Single daily oral dose	Cycle	Cycles > 4 days: control = 6%, PCB = 67.5%
Orberg and Kihlstrom, 1973 (*23*)	Clophen A60	0.8 mg/kg/day, 10 weeks	Single daily oral dose	Cycle	Days between cornified smears: control = 6.6 ± 2.5, PCB = 8.7 ± 4.3
Orberg et al., 1972 (*24*)	Clophen A60	20 mg/kg	IP, one treatment	Animal	Days between cornified smears; group means not stated
Jonsson et al., 1976 (*27*)	Aroclor 1242	2.5, 5.0 mg/kg/day, 36 weeks	In feed	Animal	Cycle length, days: control = 5.42, PCB 2.5 mg/kg = 5.34, PCB 5.0 mg/kg = 5.25

However, in a fourth study (*27*), rats were fed 3.7 or 7.5 mg/kg/day Aroclor 1242 for 36 weeks, and no effects on estrous cycles were found.

The studies reporting effects on estrous cycles differed from the study that did not find this effect in several respects, as shown in Table 3. The studies reporting the effect used agents with a greater chlorine content and administered the agent in single daily doses rather than in feed, thus possibly producing higher peak plasma levels. Also, two studies (*23,25*) reporting an effect used the individual cycles, pooled across individuals within groups, as the unit for statistical analysis. Thus, the analysis did not take into account the fact that the cycles, obtained from multiple observations in each animal, were not independent events. The study (*27*) that found no effect based the statistical analysis on the average cycle length of each subject.

Although the studies are not consistent in demonstrating effects on estrous cycles, other effects reflecting a disturbance of female reproductive function were found in each. Reduced circulating progesterone levels and distinctive histological change in ovarian stromal cells were reported in the Jonsson et al. study (*27*) at the end of the treatment period, although estrous cycle length was not affected. In the same study, there were fewer matings (vaginal plugs) after exposure to males in females given 7.5 mg/kg/day Aroclor 1242 in diet than in controls. Fewer implantation sites were reported in the Orberg and Kihlstrom study (*23*) (statistics based on implantations/corpora lutea for pooled group data). Brezner et al. (*25*) reported fewer "copulations" in treated animals than in controls (80 versus 100%). Thus, parameters other than estrous cycle suggest adverse effects on female reproductive function. The effect on estrous cycle at 0.8 mg/kg/day (*25*) could be considered the LOAEL for female reproductive function, although the statistical basis for identifying this effect is questionable, as discussed above. A NOAEL for female reproductive effects could not be identified because the LOAEL was the lowest dose tested.

Effects on Postnatal Growth and Function

PCB mixtures produce effects on postnatal measures of mortality, weight gain, reproductive organ weights, and postnatal function (including primarily neurobehavioral development but also thyroid function and reproductive performance) (Table 4). Table 4 includes all species for which data are available on postnatal end points. Confidence can be placed in this evidence for developmental toxicity because the effects are replicable; they have been demonstrated in several species and in the same species in independent studies.

The end points in Table 4 can be considered to reflect developmental toxicity, although it is difficult to determine the time of insult responsible for the effects. Typically, PCBs were administered chronically in maternal diet throughout gestation and lactation so that exposure via nursing sometimes occurred at the same time that end points were being measured. Some effects, such as reduction in spleen weights, alterations in electroshock seizure patterns (*29*), and cognitive defects (*52,53*), were detected after discontinuation of dosing, but the persistence of body burden makes it difficult to determine when the biological action responsible for the effects took place.

One study of postnatal neurobehavioral end points (*49*) used a fostering design so that pups exposed *in utero* (day 6 to 15 gestation) were nursed by unexposed mothers. Neurobehavioral effects were seen in offspring up to 30 days of age, the oldest age tested. This study indicates that exposure to PCB mixtures during a limited period of prenatal development may have long-lasting effects. In other studies, it is possible only to state that PCBs continuously present in maternal diet during gestation and lactation can disrupt normal patterns of development.

A similarity in PCB-induced effects between adult and developmental studies is indicated. PCB mixtures are known to affect weight gain and thyroid function in adults (*58,59*), as is the case in developing organisms (Table 4). Immunotoxicity, which has been reported in adult PCB toxicity syndromes, has not been reported after developmental exposure in mice (*60*) and rabbits (*46*). In mice, there were no effects on spleen weights and histopathology, delayed type hypersensitivity, *in vitro* phagocytosis, or humoral immunity in the offspring at weaning (*60*). In rabbits, there were no effects on spleen and thymus weights and histopathology, mitogen-stimulated lymphocyte proliferation, or humoral immunity, although a lower contact sensitivity response was reported at the highest dose (250 ppm in diet) (*46*). Immune function was not included in Table 4 because the results are generally negative for this end point.

A LOAEL of 0.1 mg/kg/day was identified for effects on rodent postnatal growth and neurobehavioral development (*29*) (Table 4). The effects at this dose level were not permanent. Specifically, growth was retarded at day 14 but not day 21 postnatal, and the percentage of animals demonstrating auditory startle was lower on day 12 but not days 13 and 14. At higher doses (1.0 mg/kg/day), long-term changes of the same type were seen, and dose-response relationships were indicated at the ages where the lower dose was effective. This indicates that the LOAEL based on transient effects is a valid indicator of developmental toxicity (*16*). NOAELs for developmental toxicity in rodents could not be identified, as the LOAEL was the lowest dose tested.

Table 4. LOAELs and NOAELs for postnatal developmental end points.[a]

	Ages evaluated[b]	Agent/species	Doses tested, mg/kg/day	Size of effect (% change from control)	LOAEL, mg/kg/day	NOAEL, mg/kg/day	Reference
Body weight	0,7,*14,21*,150 days	Aroclor 1254/rats	0.1,1,17	8	0.1 (day 14 only)	None	(*29*)
	0,*4,8,12* weeks	Aroclor 1254/mice	1.3	12–15	1.3	None	(*42*)
	1,2,3–4 months	Aroclor 1254/rats	10	64,47,19	10.0	None	(*25*)
	1,*3,5* weeks	Aroclor 1254/mink	0.1	20	0.1	None	(*45*)
	0,7,14,*21* days	Aroclor 1254/rats	2.5,25	7,13	2.5	None	(*37*)
	3–9 weeks	Aroclor 1248/rabbits	0.44,4.4,11	16	11.0	4.4	(*46*)
	0 days	Aroclor 1248/rhesus monkeys	0.09,0.20	21	0.09	None	(*40*)
	0 days	Aroclor 1016/rhesus monkeys	0.008,0.030	18	0.028	0.008	(*41*)
Relative organ weight[c] male accesory glands	*8,12* weeks	Aroclor 1254/mice	1.3	29,44	1.3	None	(*42*)
Uterus	*8,12* weeks	Aroclor 1254/mice	1.3	28,35	1.3	None	(*42*)
Ovaries	*8,12* weeks	Aroclor 1254/mice	1.3	27,39	1.3	None	(*42*)
Thymus	*21*,150 days	Aroclor 1254/rats	0.1,1,17	35	1.3	0.1	(*29*)
Spleen	21,*150* days	Aroclor 1254/rats	0.1,1,17	10	—[d]	—[d]	(*29*)
Impaired function							
Thyroxine/tri-iodothyrodine production	0,*7,14,21* days	Aroclor 1254/rats	2.5,25	60	2.5	None	(*37*)
Electroshock seizure patterns	40,*100*,150,250, 330 days	Aroclor 1254/rats	0.1,1,17	30	—[d]	—[d]	(*29*)
Swimming ontogeny	6–14 days	Fenclor 42/rats	1,2,4,5,10	90	1	None	(*49*)
Cliff avoidance	*4,6,8,10* days	Fenclor 42/rats	1,2,4,5,10	90	1	None	(*49*)
Negative geotaxis	5,6,*7,8* days	Aroclor 1254/rats	0.1,1,17	60	1.0	0.1	(*29*)
Auditory startle	11,*12*,13,14 days	Aroclor 1254/rats	0.1,1,17	33	0.1	None	(*29*)
Air righting	16,17,*18*,19 days	Aroclor 1254/rats	0.1,1,17	10	1.0	0.1	(*29*)
Open field activity	*14,21* days	Fenclor 42/rats	1,2,4,5,10	90	2	1	(*49*)
Open field activity	*6,12,44* months	Aroclor 1248/rhesus monkeys	0.09	Not recorded	0.09	None	(*51*)
Open field activity	12 months	Aroclor 1248/rhesus monkeys	0.006,0.013	Not recorded	None	0.013	(*51*)
Spatial and shape discrimination	14 months	Aroclor 1016/rhesus monkeys	0.008,0.030	Not recorded	0.03	0.008	(*52*)
Maze learning	*13* weeks	Kanechlor 500/rats	20	60	—[d]	—[d]	(*31*)
Active avoidance	*30* days	Fenclor 42/rats	1,2,4,5,10	30	2	1	(*49*)
Active avoidance	*23* days	Aroclor 1254/mice	0.5,4.1	15	—[d]	—[d]	(*50*)
Reproductive performance	Continuous breeding	Aroclor 1254/mice	1.3	20	1.3	None	(*42*)
Reproductive performance	*130–150* days	Aroclor 1254/rats	8,32,64	50	32	8	(*48*)

[a]All species are included for which postnatal data are available.
[b]Ages at which group differences were identified are italicized.
[c]Expressed as percent body weight or milligrams per gram body weight.
[d]Not dose dependent.

Table 5. Comparison of LOAELs for postnatal end points by species.

Species	Agent	LOAEL, ppm	LOAEL, mg/kg/day[a]	LOAEL, $(mg/kg)^{2/3}$/day	Reference
Mouse	Aroclor 1254	10	1.3	0.364	(*43*)
Rat	Aroclor 1254	2.5	0.25	0.167	(*29*)
Mink	Aroclor 1254	1.0	0.115	0.115	(*45*)
Monkey	Aroclor 1016	0.25	0.008	0.017	(*53*)

[a]LOAEL mg/kg/day = ppm × F/W (*61*), where ppm = mg PCB/kg diet; F = food intake/day; W = g body weight. Information of F and W: for mice (*42*); for rats (*29*); for mink (*45*); for monkeys (*63*).
[b]LOAEL $(mg/kg)^{2/3}$/day = LOAEL mg/kg × $W^{1/3}$ (*61*). For mice W = 23 g (*42*); for rats W = 300 g (*29*); for mink W = 1 kg (*45*); for monkeys W = 10 kg.

Identifying NOAELS: Sensitive Species

Table 5 compares LOAELs for the most sensitive end points (postnatal growth and function) in species for which data are available (rats, mice, mink and nonhuman primates). Mink and monkeys are generally more sensitive to the toxic effects of commercial PCB mixtures (on a milligram per kilogram basis) than are rats and mice. Increased dam mortality in developmental experiments is seen at 50 mg/kg/day in rats, 1 mg/kg/day in mink, and 0.2 mg/kg/day in rhesus monkeys.

For Table 5, information on body weight and food intake from

each experiment was used to derive more precise estimates of milligram per kilogram dosages from dietary PCB concentrations. In addition, LOAELs in different species are compared on both a milligram per kilogram and a milligram per unit body surface area ([milligram/kilogram]$^{2/3}$) basis.

Although mink are clearly very sensitive to PCB toxicity, the LOAEL for reproductive effects (0.1 mg/kg/day based on postnatal growth retardation) was similar to that identified for rats and mice. Notably, total reproductive failure occurred in mink at 0.2 mg/kg/day, only slightly above the LOAEL. Further, the effect on growth retardation at the LOAEL was substantial, and lower doses were not tested. Because low dose ranges and sensitive end points have not been examined in mink studies, it is difficult to determine whether they are more sensitive than rats and mice to developmental toxicity.

Although both reproductive and maternal toxicity occur over a small dose range in mink, there is evidence that reproductive toxicity occurs at levels below those producing maternal toxicity. As plotted in Figure 3, dose-response curves for reproductive failure (kits born per female) are steeper than for female mortality (females per group surviving).

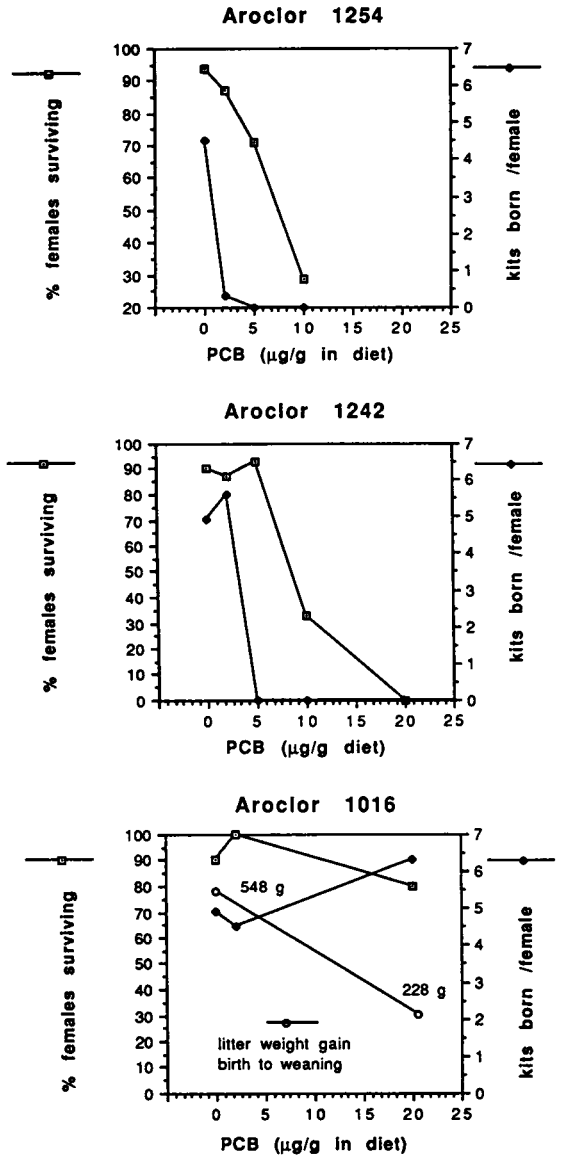

FIGURE 3. Dose-response curves for general toxicity and reproductive failure in mink fed Aroclor 1254, 1248, or 1016 in diet throughout one breeding season. Data from Bleavins et al. (*28*).

Nonhuman primates also appear to be affected by PCB toxicity at lower doses than are rodent species. Developmental LOAELS were seen at the lowest dose levels investigated in any species (0.008 mg/kg/day, 0.25 ppm in diet). In the case of rhesus monkeys, sensitive postnatal end points were examined in detail. A NOAEL for transient growth retardation and postnatal behavioral deficit was identified at 0.008 mg/kg/day; however, a characteristic toxic sign (skin hyperpigmentation) was reported at this dose, and this dose is included as a LOAEL in Table 5.

As presented in Table 5, LOAELs are more similar across species when compared on a milligram per body surface area basis. The LOAEL derived from nonhuman primate studies may be the lower because of the end point, the mixture used, the dose range explored, or a species difference in sensitivity.

Notably, the LOAELs in Table 5 were from studies that used different PCB mixtures, Aroclor 1016 and 1254 (41 and 54% chlorine, respectively). There is no clear basis in the current literature for separating PCB mixtures with 41 to 54% chlorine for derivation of NOAELs and LOAELs. NOAELs and LOAELs for mixtures outside of this range of chlorination are difficult to derive because of the limited number of studies available. The LOAEL for studies using Aroclor 1260 (60% chlorine), if considered separately, would be 25 mg/kg/day ([0.83 mg/kg]$^{2/3}$/day) based on postnatal survival in a multigeneration study in rats (*36*). The NOAEL for Aroclor 1260 is 5 mg/kg/day ([0.60 mg/kg]$^{2/3}$/day) based on the same end point in the same study. The LOAEL for Aroclor 1221, if considered separately, would be 1000 mg/kg/day ([42.9 mg/kg]$^{2/3}$/day) based on disruption of female reproductive function in mice after neonatal treatment. The NOAEL for Aroclor 1221 would be 1 mg/kg/day ([0.07 mg/kg]$^{2/3}$/day) based on failure to affect maternal weight gain and litter size in rabbits (*37*). Less confidence can be placed in the separate LOAELs and NOAELS for Aroclor 1260 and 1221 because they are based on a very few studies compared to the LOAELs for the 41 to 54% mixtures. Studies of sensitive postnatal end points (growth and function) have not been carried out for Aroclor 1260 and 1221. The LOAEL for the 41 to 54% mixtures could be applied to these mixtures.

Accurate estimation of no-effect levels for commercial PCB mixtures will require exploration of lower dietary PCB levels than have been studied thus far. To undertake such studies at lower dose levels (< 0.25 ppm), background contamination of standard (control) diets with PCBs must be considered. In reproductive toxicity studies reviewed here control diets contained 0.005 to 0.9 ppm PCB [for monkeys 0.005 ppm (*41*), for mink, 0.3 ppm (*64*), for rats, 0.02 ppm (*29*) and 0.9 ppm (*25*), for mice 0.2 ppm (*50*)]. Commercial laboratory feeds can contain 0.01 to 0.03 ppm (*65*). Given individual variability in food intake, PCB exposure of treated and control animals could overlap at these low dose levels unless purified diets free of background PCB contamination are used.

Identifying LOAELs: Quality of Studies

The LOAELs identified for nonhuman primates and rodents for Aroclor 1016 and 1254 were derived from recent studies of

good quality (*29,53*). Diets were analyzed to confirm PCB levels. In addition, tissue analyses were presented to support treatment group differences in exposure. Dose-response designs (at least two dose levels and control) were used along with appropriate statistical analysis. The study by Overmann (*29*) also included a minimally maternally toxic dose and assessed maternal toxicity.

Supporting Evidence

Replicability and Species Concordance. Replicability within and across species is an important factor in evaluating an experimental finding that provides the basis of NOAELs and LOAELs. The general findings on which the rodent and nonhuman primate LOAELs are based (postnatal manifestations of chronic dosing during gestation and lactation) have been reported in several studies in rats and one study in mice, as well as in the monkey study.

Biological Plausibility. PCB effects on growth have been ascribed to interference with thyroid function, which is characteristic of these agents in both adults and immature animals (*37,66*). The fact that low doses affect primarily postnatal end points may be attributed to mobilization of PCBs from fat stores during lactation and subsequent transfer during nursing (*67*). Effects on behavior may be related to changes in central nervous system neurotransmitter levels produced by PCB mixtures and individual PCB congeners (*68*).

Effects on menstrual/estrous cycle, reduced conceptions, and altered weights of offspring reproductive organs may be related to estrogenic effects of PCBs. Individual PCB congeners with lower chlorination (1–3 chlorines) exhibit structure-specific estrogen receptor binding and PCB mixtures with a greater percentage of these congeners are weakly estrogenic in acute situations (*69*). Hormonally mediated effects in males or females might also be caused by altered metabolism of endogenous steroids via hepatic enzyme induction (*70*).

Relevance to humans. The end points on which LOAELs for rodents and nonhuman primates are based (growth retardation, neurobehavioral developmental delay, hyperpigmentation) have all been associated with PCB exposure in humans during development (*71–74*). In addition, growth retardation and developmental delay in humans have been associates specifically with PCB exposures that occur via the food chain (*75*).

Route of exposure in animal studies (via diet) is relevant to the typical human route of exposure. However, because animal studies use direct dosing with commercial mixtures, they do not resemble the most common type of human dietary exposure, which occurs via contamination of the food chain. There is little information on effects in humans from direct exposures to commercial mixtures. PCB mixtures that persist in the environment and animal tissues are known to differ in composition from the original commercial mixtures that led to the contamination. Animal studies, however, indicate that exposure via the food chain (*64,76,77*) has similar effects to dosing with commercial mixtures.

Summary

Studies in animals indicate a continuum of reproductive effects of commercial PCB mixtures from the highest to the lowest doses studied. At the lowest doses, developmental exposures to commercial PCB mixtures have been shown to produce postnatal effects on growth and function in several species. The LOAEL for these effects can be identified at 0.25 mg/kg/day ([0.167 mg/kg]$^{2/3}$/day) for rodents and 0.008 mg/kg/day ([0.017 mg/kg]$^{2/3}$/day) for nonhuman primates. These LOAELs are based on good quality, replicable findings in several species as well as information supporting biological plausibility and human relevance. Research needs include identification of a NOAEL for sensitive end points (only a LOAEL could be obtained from existing literature); studies that better identify sensitive exposure periods during development; and investigation of the basis for the enhanced sensitivity of postnatal end points. Also, studies that identify the congeners or combinations of congeners that produce specific effects would be helpful in generalizing from animal studies to human exposures.

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