Immunotoxicity of PCBs (Aroclors) in Relation to Great Lakes

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Polychlorinated biphenyls (PCBs) are among the most widespread environmental pollutants and a prominent contaminant of the Great Lakes basin. Due to their resistance to biodegradation and lipophilic properties, PCBs bioaccumulate in fish tissues and in fish-eating humans. PCBs are also known to cross the placenta and to be excreted into the mother's milk, thus predisposing the infant to potentially adverse health effects. For example, a higher incidence of bacterial infections was reported for breast-fed infants born to mothers who consumed large amounts of Great Lakes fish compared to the incidence in control infants whose mothers ingested low amounts of fish. While data regarding the PCB-induced immunotoxic effects in humans are scarce, data derived from the use of experimental animals, including nonhuman primates, indicate that the immune system is a potential target for the immunotoxic effects of PCBs. Such studies have used the commercially available PCB mixtures alone. However, PCBs have the potential of partially antagonizing the effects of other structurally related compounds including the highly toxic dioxins, which are also present in small amounts in the Great Lakes. Thus, to fully evaluate the magnitude of the immunotoxic risk PCBs pose to humans, consideration should be given to investigations in which the interactive effects of PCBs are combined with other contaminants present in the Great Lakes. — Environ Health Perspect 103(Suppl 9):35–46 (1995)

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Introduction

Commercial polychlorinated biphenyls (PCBs) are ubiquitous chemical mixtures containing many of the 209 possible congeners (*1*). Their widespread use for diverse industrial purposes during the last 60 years led to considerable contamination of most ecosystems throughout the world (*2,3*).

Due to their resistance to decomposition in the natural environment, PCBs persist in the environment and are able to migrate widely through natural atmospheric and water transport mechanisms. Although PCBs are only slightly soluble in water, they dissolve readily in oils and are thus accumulated in the fatty tissues of fish, birds, animals, and humans (*4,5*). PCBs are one of the major contaminants of the Great Lakes and surrounding streams. Humans are exposed to the Great Lakes contaminants through the consumption of food, primarily fish, and the ingestion of drinking water (*6–8*). It has been reported that consumption of fish from Lake Michigan correlated with PCB levels in human maternal serum and milk (*9*). These data suggested that certain populations, including people who fish, their families, and native Americans, who consume large amounts of Great Lakes fish might be at risk for PCB-related health effects (*9,10*). For example, epidemiologic studies of Great Lakes fish-eating cohorts have shown that breast-fed infants of mothers who consumed high amounts of fish had a higher incidence of microbial infections than breast-fed infants of mothers who did not eat fish (*11,12*).

In addition to these studies, there is evidence which indicates that PCBs may affect the survival of a variety of living organisms such as birds (*3*), seals (*13*), and beluga whales (*Delphinapterus leucas*) (*13*). This has raised serious concerns among government regulators and the public at large regarding the safety of Great Lakes water and fish. These concerns have been addressed over the years by several regulatory agencies at national and international levels, and guidelines have been issued regarding safe levels of PCBs in the Great Lakes water and fish (*14–16*). Such guidelines were based mainly on carcinogenicity end points and have not taken into consideration the potential adverse immunotoxic effects of PCBs. While the effects of PCBs on the immune system of humans have not been studied systematically, data derived from studies whereby laboratory animals and nonhuman primates were exposed to PCBs indicate that the immune system is perhaps the most sensitive target for PCB induced toxicity (*17–20*).

The objective of this paper is to critically review the experimental and human epidemiologic data regarding effects of PCBs on the immune system to identify any limitations inherent to the existing immunotoxicity data and to suggest additional data requirements that might contribute to the determination of the magnitude of risk that PCBs, present in the Great Lakes, pose to human health.

Mechanisms of Immunotoxicity

The mechanism(s) by which PCBs induce their immunotoxic effects has not been completely elucidated. Extensive studies on structure–receptor binding relationships, structure–induction relationships, and structure–toxicity relationships revealed that several PCBs may share a common mechanism of action with other structurally related halogenated aromatic hydrocarbons such as the tetrachlorodibenzo-*p*-dioxins (TCDD) and chlorodibenzofurans (CDF) (*21*). Such studies demonstrated that some of the immunotoxic effects for the dioxinlike

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Abbreviations used: Ah, aromatic hydrocarbon; BSA, bovine serum albumin; CDF, chlorodibenzofurans; ConA, concanavalin A; Cr, Credit River; DNP, dinitrophenol; DTH, delayed-type hypersensitivity; GVH, graft-versus-host; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; NK, natural killer; OS, Owen Sound; PCBs, polychlorinated biphenyls; PCDFs, polychlorinated dibenzofurans; PCQ, polychlorinated quaterphenyls; PFC, plaque-forming cell; PHA, phytohemagglutinin; PMS, phorbol myristate acetate; PWM, pokeweed mitogen; SRBC, sheep red blood cells; STM, *Salmonella typhimurium* mitogen; TCB, tetrachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEFs, toxic equivalency factors; tt, tetanus toxoid; WBC, white blood cells.

PCB congeners depend on the presence of the aromatic hydrocarbon (Ah) receptor, which has been shown to regulate the synthesis of a variety of proteins (*21,22*).The presence of the Ah receptor in tissues and the ability of PCBs to bind to this receptor is a prerequisite for the observed immunotoxic effects of the dioxinlike PCBs (*22,23*). The affinity of PCBs for the Ah receptor depends on their molecular conformation, which is determined by the chlorine substitution pattern (*24*). Thus, the binding affinity to the Ah locus is found to be greatest for the more toxic PCB congeners, 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta-, and 3,3′,4,4′,5,5′-hexa-chlorobiphenyls (*25*). With respect to immunotoxicity, it has been shown that the coplanar 3,3′,4,4′-tetrachlorobiphenyl (TCB), which binds the receptor with a relatively high affinity, causes severe suppression of the humoral antibody response in C57Bl/6 (Ah^b/Ah^b) mice but not in the genetically different DBA/2 D2 (Ah_d/Ah_d) mice in which TCB exhibits a lower binding affinity for the Ah receptor (*24*). In contrast, the di-*ortho*-substituted 2,2′,5,5′-TCB, which is predominantly in a nonplanar configuration has weak receptor-binding affinity, and hence is not immunosuppressive in either mouse strain (*24*).

Mechanisms other than those involving the Ah receptor may be operable in some of the PCB congener-induced immunotoxicity. Such effects are thought to be mediated via metabolism to arene oxide intermediates capable of alkylating critical cellular macromolecules to form potentially toxic covalently bound substrate-macromolecular adducts (*26*). Differences in the mechanisms by which PCBs induce immunotoxicity are significant when attempts are made to calculate toxic equivalency factors (TEFs) for PCBs (*22*).

Animal Studies (Rodents, Rabbits, Guinea Pigs, and Chickens)

The commercial PCB mixtures reaching the environment may contain as many as 209 PCB congeners. Many of these mixtures have been synthesized in the laboratory and are readily available for research purposes. Consequently, studies regarding the immunomodulating properties of PCBs have been carried out using commercially available PCB mixtures. PCB mixtures are known by a variety of trade names depending on the country of production: Aroclor (United States), Phenochlor (France), Clophen (Germany), Kanechlor (Japan), Fenchlor (Italy), and Sovol (Union of Soviet Socialist Republics). Aroclors are identified by a four-digit number. The first two digits refer to the 12 carbon atoms and the last two digits refer to the percent, by weight, of chlorine in the mixture. Thus, Aroclor 1260, contains about 60% chlorine, while Aroclor 1254 contains about 54% chlorine (*25*).

Recent studies have demonstrated that the immunologic response to sheep red blood cells (SRBC) is a sensitive indicator for predicting the immunotoxic potential of chemicals (*27*). This parameter has been used to establish immunologic dose–response relationships of several Aroclors in mice exposed to a single ip injection of the PCB congeners (*28*). Such studies indicated that the higher chlorinated PCB mixtures of Aroclors 1260, 1254, and 1248 are more immunotoxic than the lower chlorinated Aroclors 1232, 1016, and 1242. At the lowest dose, 50 mg/kg body weight (bw), both Aroclor 1260 and 1254 significantly reduced the plaque forming cell (PFC) response, whereas the lower chlorinated Aroclors 1248, 1242, 1016, and 1232 were significantly immunotoxic only at levels higher than 50 mg/kg bw (Aroclor 1248 at a dose of 150, and Aroclor 1242, 1016 and 1232 at a dose of 450 mg/kg bw). The calculated ED_{50} values (mmol/kg bw) were as follows: Aroclor 1260 > 1254 > 1248 > 1242 > 1016 > 1232 (*28*). Additional data in experimental animals confirmed these conclusions (*14*). A review of the available data for each of the commercially available PCB mixtures is presented below.

Aroclor 1260. Limited information exists on the potential immunomodulating effects of Aroclor 1260 following oral or dermal exposure (*29–33*). A dose level of 400 ppm of Aroclor 1260 administered in feed for 60 days resulted in decreased spleen weights and eventual mortality in chickens (*29*), while a sublethal dose level of 50 ppm administered to female guinea pigs for 6 weeks resulted in profound depressive effects on the function of humoral and cellular parameters of the immune system including reduced antibody titers to tetanus toxoid (tt) and decreased leukocyte and lymphocyte counts in peripheral blood. The observed effects were not related to stress due to the release of glucocorticosteroids by the adrenal glands but were thought to be the result of a direct effect of PCBs on the immune system (*30*). Similarly, in the study by Vos and de Roij (*31*), dose levels of 10 and 50 ppm of Aroclor 1260 were administered to guinea pigs in the feed for 8 weeks; this resulted in a number of immunomodulatory effects, most of which were not dose related. These included a decrease in gamma globulin-containing cells in popliteal lymph nodes following stimulation of the foot pad with tt in the 10- and 50-ppm groups; an increase in the percent serum albumin in the tt-stimulated and -unstimulated guinea pigs; a decrease in percent gamma globulin at the 10 ppm group only; a decrease in cervical lymph node weights in the 10-ppm group only; and an increase in the mesenteric lymph nodes at both levels. However, the leukocyte counts were unaffected by the PCB treatment.

The effects of Aroclor 1260 have also been investigated by Vos and Beems (*32*) and Vos and Notenboom-Ram (*33*) in rabbits following dermal exposure. In these studies, doses of 118 (*32*) or 120 mg/kg bw/day (*33*) were applied to the skin of female rabbits for 38 days or 28 days, respectively. Unequivocal thymus atrophy of the cortex was reported in both studies, but conflicting results were obtained with respect to histopathology of spleen and lymph nodes, as well as effects on body weights. Specifically, the 118-mg dose level resulted in reduced germinal centers in the spleen and lymph nodes and in decreased body weights (*32*), while the 120 mg/kg bw/day dose did not have any effect on these parameters (*33*).

Aroclor 1254. In contrast to Aroclor 1260, the immunomodulating effects of Aroclor 1254 have been studied in rodents and in rabbits by a number of investigators (*34–45*).

In the experiment by Wierda et al. (*34*), Aroclor 1254 was injected into male mice ip for 4 days at doses of 550, 250, 135, and 63 mg/kg bw/day; this resulted in a decreased PFC response to SRBC and in decreased blood leukocyte numbers at 550-, 250-, and 135-mg/kg bw/day doses but not at 63 mg/kg bw/day; a decrease in spleen cellularity was noted in all doses. The concanavalin A-induced (ConA) and lipopolysaccharide-induced (LPS) mitogenic response of splenocytes was not affected by treatment.

Smith et al. (*35*) studied the effects of acute peroral (gavage) or subacute parenteral (sc injection for 14 days) exposure of adult and neonatal mice to Aroclor 1254 at doses of 0.3 to 75 mg/kg bw and found that the ability of mice to clear *Listeria monocytogenes* from the circulation was reduced in adult and neonatal males at the 0.3-mg/kg dose and in adult and neonatal

females at the 75-mg/kg dose, indicating a greater sensitivity to PCB treatment of adult and neonatal males relative to that of females. Similarly, an increased sensitivity to Moloney leukemia virus was noted in male mice fed 3.75, 37.5, or 375 ppm for 6 months (*36*).

The observed lower sensitivity of female mice to Aroclor 1254 exposure was also reported by Talcott and Koller (*37*). In this study, doses of 1.17, 11.67, or 29.17 mg/kg bw/day (10, 100, or 250 ppm) of Aroclor 1254 administered to female mice in feed for 12 weeks did not have any significant effect on body weights or antibody titers to bovine serum albumin (BSA), phagocytosis measured by ingestion of SRBC by peritoneal macrophages *in vitro*, or delayed-type hypersensitivity to Oxazolone. The only notable effect was a decrease in spleen weights relative to body weights at the 250 ppm dose level.

Exposure of male rats to Aroclor 1254 in feed also resulted in a number of significant effects on humoral and cell-mediated immune parameters. Talcott et al. (*37,38*) and Exon (*39*) fed Aroclor 1254 at doses of 50 or 500 ppm to male rats for 10 weeks. A decrease in serum immunoglobulin G (IgG) antibody titers to keyhole limpet hemocyanin (KLH) antigen was observed at both dose levels, whereas the interleukin 2 (IL-2) production by ConA-stimulated splenocytes was increased at these levels. On the contrary, IL-2 production by ConA-stimulated splenocytes was decreased when cells were exposed *in vitro* to 0.4 or 20 µg/ml of Aroclor 1254 (*38*). The natural killer (NK) cell activity was decreased at both dose levels and in the *in vitro* exposure of splenocytes to 0.4 or 20 µg/ml of Aroclor 1254 (*38*).

Depressed NK activity was also noted by Smialowicz (*40*) following repeated treatment with Aroclor 1254 at doses of 10 and 25 mg/kg bw/day administered to male rats orally (gavage). Doses of 0.1 or 1 mg/kg bw/day had no effect on NK cell activity. The effect on NK cell activity by the higher dose levels was accompanied by decreased body and thymus weights. Increased phytohemagglutinin (PHA) but not ConA- , pokeweed mitogen (PWM)-, or *Salmonella typhimurium* (STM)-mitogen-induced lymphocyte proliferation was noted at the 25-mg/kg bw/day dose levels. Similarly, increased PHA- but not PWM-induced lymphoproliferative activity was observed in male rats fed 1.88 mg/kg diet/day (25 ppm) of Aroclor 1254 for 7 days (*41*). These results suggest that Aroclor 1254 affected a subset of T-lymphocytes rather than the B-lymphocytes. In contrast to the increased sensitivity of male mice and rats to Aroclor 1254, male rabbits administered Aroclor 1254 at doses of 0.18, 0.92, 2.10, or 6.44 mg/kg diet/day (3.7, 20.0, 45.8, or 170.0 ppm) for 8 weeks did not show any effect on antibody levels to SRBC, the gamma globulin/transferrin ratio, or skin reactivity to tuberculin, albeit thymus atrophy was observed across all dose levels (*42*).

The role that Aroclor 1254 plays in tumor development is not clear. Paradoxically, the available data reveal a protective effect of sublethal but immunotoxic doses of this mixture. The experiments by Kerkvliet and Kimedorf (*43,44*), whereby the effect of Aroclor 1254 on the transplantability and growth of the Walker 256 carcinosarcoma in rats was studied, showed that growth of the tumor cells was retarded in rats treated with single ip injections of 100 or 200 mg/kg bw compared to control rats. Experiments were based on the hypothesis that a suppressed immunologic state in the host can enhance the generation and growth of tumors. At these dose levels of PCB, increased resistance to transplanted tumors, decreased metastases, increased incidence of tumor regression, and increased survival time of rats compared to control (non-PCB-treated but tumor-transplanted rats) were observed. Lethality was observed in 9/16 rats only after exposure to a high dose of 400 mg/kg bw of Aroclor 1254.

The protective effect of sublethal levels of Aroclor 1254 was also noted in groups of mice fed doses of 10, 50, or 250 ppm. A decreased mortality rate compared to the control group was observed in the PCB-treated mice following injection with Ehrlich's tumor ascites cells (*45*). Similarly, BALB/c male mice fed Aroclor 1254 at sublethal doses of 3.75 or 37.5 ppm for 6 months and subsequently injected with Moloney leukemia virus did not differ in the incidence and severity of hepatic lesions from that in the control group, while a high dose of 375 ppm resulted in 92% lethality and increased incidence and severity of hepatic lesions (*36*).

Aroclor 1248. Thomas and Hinsdill (*46*) investigated Aroclor 1248 at doses of 50, 100, 500, and 1000 ppm fed to female mice for 3 to 5 weeks. The spleen and thymus weights were not affected by treatment. Resistance to *S. typhimurium*, examined only at 1000 ppm, was decreased compared to the control group, and endotoxin sensitivity examined at 100 or 1000 ppm was increased over the control values. The antibody response to SRBC examined at the 1000-ppm dose was not affected by treatment.

In subsequent studies, Thomas and Hinsdill (*47*) investigated the effect of Aroclor 1248 administered to adult female rabbits (three animals per group) at levels of 10, 100, or 250 ppm for 4 weeks. These were mated and the offspring continued to be exposed to PCBs in milk until they were weaned at 4 weeks of age. No effect was noted in the adult weight gain, litter size, or postnatal mortality. In offspring, weaned at 4 weeks of age and tested at 7 weeks of age, contact sensitivity to dinitrofluorobenzene was reduced only at the 250-ppm dose level compared to controls. At that level, the body weight of the exposed offspring was lower than that of the control group, making it difficult to determine whether the observed effect on delayed-type hypersensitivity was direct due to PCB treatment or secondary to some other form of toxicity. No treatment-related effect was detected for the thymus and spleen weights, PFC numbers, anti-SRBC antibody levels, or the mitogen-stimulated lymphocyte proliferation, indicating that the humoral and cellular immune functions associated with these parameters were not affected by the PCB treatment.

Aroclor 1242. A single ip injection of Aroclor 1242 at 1000 mg /kg bw to male mice resulted in spleen enlargement with decreased absolute lymphocyte counts. A transiently increased graft-versus-host (GVH) reaction was also observed in treated mice compared to controls (*48*).

Functional studies using a single clinically subtoxic dietary dose level of 167 ppm of Aroclor 1242 fed to male mice for 3 and 6 weeks showed a decrease in primary and secondary PFC responses to SRBC with concomitant decreases in total serum IgG1, IgA, and IgM without any detectable effects on spleen and thymus weights or histopathological changes in the thymus, spleen, and mesenteric lymph nodes (*49–52*). It is not clear from these studies whether antibody memory cell function was impaired or whether cell–cell cooperation was affected. At the same dose and length of exposure, an increased susceptibility to bacterial endotoxin (*Salmonella typhosa*) and the malaria parasite *Plasmodium berghei* was noted. However, this dose level of Aroclor 1242 fed to male mice for 18 weeks did not have any detectable effect on macrophage functions such as oxygen consumption and

phagocytic or microbiocidal activity (*53*). Since macrophages play a crucial role in combating infection, it is possible that macrophage functions other that those listed above may have been affected by the treatment. In fact, RNA synthesis by elicited mouse peritoneal macrophages tested *in vitro* was inhibited by 22.6, 25.0, or 28.3 µg/ml of Aroclor 1242; coincubation of macrophages with PCB dose levels of 1.0 or 7.25 µg/ml had no effect (*54*).

Like Aroclor 1254, doses of 5 or 100 ppm of Aroclor 1242 fed to mice for 3, 6, or 18 weeks did not affect resistance to tumor challenge by EL-4, MKSA, and P388 tumors or tumoricidal activity of the animals albeit cytotoxic activity of the adherent spleen cells, which represent the mononuclear phagocytic cells, was reduced in mice fed the 13.17-mg/kg (100 ppm) dose level (*53*).

Further studies in mice fed Aroclor 1242 at dose levels of 37.5 or 375 ppm for 6 months and subsequently challenged with Moloney leukemia virus resulted in an increased incidence and severity of hepatic lesions; a dietary dose level of 3.75 ppm had comparable effects on hepatic lesions to those observed in the control animals, which were challenged with Moloney leukemia virus only without exposure to Aroclor 1242 (*36*). In contrast to Aroclor 1242, Aroclor 1221 at dose levels of 3.75, 37.5, or 375 ppm fed to mice for 6 months produced hepatic lesions induced by Moloney leukemia virus, which did not differ in severity from those in the control animals injected only with the Moloney leukemia virus, confirming the comparatively lower toxicity of Aroclor 1221 (*36*).

Finally, Allen and Abrahamson (*55*) reported that the body-weight gain, thymus weight, and the white blood cell (WBC) and neutrophil counts were decreased in Sprague-Dawley rats fed Aroclor 1248, 1254, and 1262 for 1, 4, and 6 weeks at a level of 0.1% of the diet; the degree of severity was 1248 > 1254 > 1262. No effect was noted on spleen weights; however, functional aspects of the immune system were not investigated.

Aroclor 1016. As predicted from the studies of Davis and Safe (*28*), Aroclor 1016 was not found to be immunotoxic at doses below 450 mg/kg. A dose level of 167 ppm fed to male mice for 40 weeks did not produce any effects on spleen and thymus weights, the GVH reaction, the mixed lymphocyte response, the mitogen-induced lymphocyte proliferation of splenocytes, or the cytotoxicity of splenocytes (*56*). However, the effects of Aroclor 1016 on humoral immunity were not investigated in this study.

Clophen A60. Vos and van Driel-Grootenhuis (*30*) investigated the effect of Clophen A60 at dose levels of 10, 50, and 250 ppm fed daily to female guinea pigs for 4 weeks. Eighty percent lethality was observed at the 250 ppm dose. Skin reactivity to tuberculin as well as antibody titers (IgM and IgG) to tt were decreased at a dose level as low as 50 ppm of PCBs. Relative thymus weights and leukocyte and lymphocyte counts were also decreased at that level.

Kanechlor 500. Imanishi et al. (*57*) reported that mice fed Kanechlor 500 at doses of 200 or 400 ppm of feed for 21 days resulted in an increased mortality rate in herpes simplex virus-infected mice. No such effect was noted at 100 ppm. The effects of Kanechlor 500 were also studied in mice exposed as adults or *in utero* (*58*). The adult female mice gavaged with 50 mg/kg bw 2 times/week for 3 weeks did not exhibit any effects on body and spleen weights, spleen cellularity, or the antidinitrophenol (DNP)–IgM PFC numbers, but a transient decrease in T-helper cell activity in the secondary anti-DNP–IgG response (adoptive cell transfer) was detected at 4 and 7 weeks but not at 11 weeks after exposure. This effect was also noted in the *in utero*-exposed offspring at 4 weeks but not at 7 or 11 weeks after exposure.

Interaction of PCBs in Mixtures

Data regarding the effects of mixtures of PCBs or of PCB interactions with other chemicals on the immune system are scarce.

It has been reported that pretreatment of C57BL/6 male mice with a single ip dose of 550 mg/kg bw of Aroclor 1254-ameliorated benzene induced leukopenia and suppression of mitogen-induced leukocyte proliferation (*34*). This was attributed to the possible interference of Aroclor 1254 with the production of benzene metabolites because Aroclor 1254 has been shown to decrease the covalent binding of benzene-associated radioactivity in the liver and lymphoid organs of rats (*59*). Also, pretreatment with Aroclor has been shown to decrease the amount of the benzene metabolites hydroquinone and catechol in the thymus, spleen, and bone marrow of rats (*60*). However, Aroclor 1254 pretreatment did not substantially alter the reduced PFC numbers induced by either benzene or Aroclor 1254 treatment alone (*34*).

Kunita et al. (*61*) administered to male Sprague-Dawley rats the PCBs Kanechlor 400 at 1.0 mg, polychlorinated quaterphenyls (PCQ) at 1.0 mg, polychlorinated dibenzofurans (PCDF) at 10.0 µg/day, or a mixture (Mix-1) of the above in feed for 22 days. Reduced anti-SRBC titers were noted for Kanechlor 400, and PCDF with a further reduction observed for the mixture, suggesting that Kanechlor 400 and PCDF had an additive effect for the suppression of antibody titers to SRBC. On the contrary, Davis and Safe (*28*) demonstrated that various Aroclors had an antagonistic effect on 2,3,7,8-TCDD immunotoxicity measured by the PFC response to SRBC. These studies demonstrated that Aroclor 1254 antagonized the effects of 2,3,7,8-TCDD and that the degree of antagonism was similar for Aroclor 1260, 1254, and 1248, whereas Aroclor 1242 and 1232 (which bind the Ah receptor with low affinity) had no antagonistic effect at the doses used. The observed antagonistic effect of Aroclors was thought to be related to their ability to displace 2,3,7,8-TCDD from the Ah receptor protein (*62*). However, similar results were obtained when 2,2′,4,4′,5,5′-hexachlorobiphenyl and 2,3,7,8-TCDD were co-administered to C57BL/6 mice (*62*), regardless of the fact that 2,2′,4,4′,5,5′-hexachlorobiphenyl does not cause responses that are mediated through the Ah receptor (*63*).

Great Lakes Fish Studies

Great Lakes contaminants have been shown to bioaccumulate within the food chain especially in aquatic organisms, in several species of fish-eating birds (*3*), and in humans mainly through consumption of fish (*9*). The toxic effects of Great Lakes contaminants, including PCBs, on rats were studied by Villeneuve et al. (*8*). In this study, diets containing salmon from Lake Ontario and the Pacific Ocean at dietary levels of 1.25, 2.90, and 5.80% were administered to Sprague-Dawley rats in feed for 28 days. The PCB and organochlorine insecticide contaminant levels in Lake Ontario salmon were considerably higher than in the Pacific salmon. Spleen weights, bone marrow cellularity, and hematological parameters were not affected by treatment. The only notable effect was a reduction in body-weight gain in the treated groups compared to the control group. However, when these dose levels of fish were administered to rats for 13 weeks, a decrease in spleen weights was observed at a dose level of 1.45% in males only for

the Lake Ontario salmon and at a dose level of 2.90% in males only for the Pacific coho salmon. No effects were noted on body weight, food consumption, bone marrow cellularity, and hematology. A decrease in bone marrow myeloid cells was noted in male rats exposed to a dietary level of 2.90% of salmon administered for 13 weeks followed by 13 weeks on the control diet (reversibility study) (*64*). The myeloid:erythroid ratio and the lymphocyte and monocyte numbers were decreased for both sources of salmon, but in the male rats only. A second study by Cleland et al. (*65*) investigated the effect of a diet containing 33% of Lake Ontario or Pacific coho salmon on the immune system of male mice fed for 2 to 4 months. A decrease in the IgM, IgG, and IgA PFC numbers was reported for Lake Ontario coho salmon but not for the Pacific coho salmon. Cytotoxic T-lymphocyte responses decreased only at a ratio of 10:1 and not at any other ratio. No effect was noted on the lymphocyte subpopulations. The differences in effects of salmon are ascribable to differences in PCB and other organochlorine contaminant levels in the two fish-containing diets.

A recently completed study in this laboratory (manuscript in preparation) investigated the effects of diet on two generations of pups born to dams fed various levels of salmon from two sources, namely the Credit River (Cr), which runs into Lake Ontario, and Lake Huron in the region of Owen Sound (OS). The latter was assumed to contain low levels of contaminants and was included as a second control in this study. The fish-containing test diets were prepared to a constant 20 percent of protein using casein as a supplement. There was one control group (rats fed rat chow supplemented to 20% with casein) and four treated groups each consisting of 30 females and 30 males randomly selected and assigned. Treated animals and their offspring received 5.60% (CR), 22.35% (CR), 5.90% (OS) and 23.81% (OS) (wet weight) fish in their diet. Adult (F_0) rats received their respective diets for 70 days prior to mating. The immunology component of the study consisted of three phases. In phase 1, (F_1 generation), pups were exposed to fish contaminants *in utero*, through maternal milk to 21 days of age, and through diet to 13 weeks of age. Immunological testing was initiated at 13 weeks of age at which time rats were considered to be immunologically mature. In phase 2 (reversibility study), F_1 generation pups treated identically to phase 1 pups were switched to control diet at 13 weeks of treatment. Pups continued on the control diet for 3 months. Immunologic tests were initiated at the end of the 3 months. In phase 3 (F_2 generation), treatment of the F_2-generation pups was identical to that of F_1-generation pups. Immunological parameters included the enumeration of PFC numbers, measurement of the NK cell activity, resistance of pups to *L. monocytogenes* infection, and an analysis of T-lymphocyte subsets in spleen mononuclear cell suspensions using flow cytometry.

There was no conclusive evidence that the fish diets had a measurable effect on the PFC numbers, the NK cell activity, or resistance to *L. monocytogenes*.

There were also no statistically significant differences among diets for the T-cell subsets ($p > 0.05$) except for the F_2-generation males. For these rats there was evidence that in the OS-treated groups the absolute leukocyte and lymphocyte levels were higher ($p = 0.001$) than the corresponding levels in the CR treated groups. This was true also for the T helper (CD4) subset levels, indicating that the increased leukocyte numbers in the 5 and 20% OS groups in comparison to the levels in the CR groups were due to an increase in the CD4 subset of the T lymphocytes.

Immunotoxocity Studies in Nonhuman Primates

The choice of an appropriate animal model in which to study the effects of PCBs on the immune system has been an important consideration in the process of evaluating the magnitude of risk which PCBs pose to human health.

The available limited *in vivo* and *in vitro* data indicate that, in general, metabolism of PCBs in monkeys may be similar to that in humans (*66*). While the degree of human sensitivity to PCBs relative to that observed in monkeys is not well characterized, the existing phylogenetic and biologic similarities between humans and monkeys make the latter a potentially useful animal model in which the immunotoxic effects of PCBs can be investigated. In this respect, the early studies by Thomas and Hinsdill (*46*) and Hori et al. (*67*) in which the immunotoxic effects of PCBs were investigated in nonhuman primates indicated that the monkey might be more sensitive to the immunotoxic effects of PCBs than the rodent.

Following these initial observations, a series of pilot studies were designed to test for toxicity of Aroclor 1254. In the first of these studies, Aroclor 1254 was given orally to female cynomolgus monkeys for 238 days at 0.1 mg/kg bw/day (2 monkeys) and 0.4 mg/kg/day (1 monkey). A decreased antibody response to SRBC was found in all treated animals compared to controls. Both monkeys given the 0.1-mg/kg bw/day dose delivered stillborn infants. The female treated with 0.4 mg/kg bw/day delivered a live infant which she nursed. This infant also failed to respond to SRBC and died at 139 days postpartum with acute confluent bronchopneumonia (*68*).

In a second pilot study, Aroclor 1254 at 0.0 or 0.2 mg/kg bw/day (5/7 days) was given orally to female cynomolgus and rhesus monkeys (4/group); untreated monkeys served as controls (4/group) (*69*). The cynomolgus animals were necropsied after dosing for 13 months; the rhesus monkeys were necropsied 15 months after the cynomolgus. Terminally, the treated rhesus monkeys had more severe clinical signs, higher blood PCB levels, and higher adipose tissue PCB levels than the treated cynomolgus or the control monkeys. The IgM (SRBC) titer was lower in treated animals compared to controls, but statistical significance was not reached because of the small numbers of animals. Rhesus monkeys were chosen for further work because of their somewhat greater sensitivity to the effects of PCBs (*70*).

Subsequent to these studies, Tryphonas et al. (*18–20*) investigated the effects of low levels of Aroclor 1254 in adult female rhesus monkeys exposed to PCBs *in utero* and during nursing. In this chronic study, five groups of female rhesus (*M. mulatta*) monkeys (16 monkeys/group) were administered Aroclor 1254 (orally in a capsule contatining glycerol:corn oil vehicle) at 0.0, 5.0, 20.0, 40.0, or 80.0 µg/kg bw/day. Immunological effects were reported at 23 months of exposure (*18*) during which time a blood PCB pharmacokinetic equilibrium was established and at 55 months into the study (*19,20*). The blood levels of PCBs noted at 23 months of exposure, were sustained through to 55 months of exposure (Table 1).

A statistically significant dose-related decrease in antibody (IgM and IgG) titers to SRBC were observed for the primary response (at 23 months of exposure) (*18*) and for the anamnestic response (at 55 months of exposure) (*19,20*). In contrast, the antibody response to the B-dependent but T-independent pneumoccocal antigens was not significantly affected. Alterations

Table I. Blood PCB levels (Aroclor 1254) in rhesus monkeys (*M. Mulatta*).

Test group	23 Months, ppb of PCB	55 Months, ppb of PCB
Control	1	1
5 µg/kg/day	10	10
20 µg/kg/day	30	40
40 µg/kg/day	70	60
80 µg/kg/day	110	120

Data from Tryphonas et al. (*18–20*).

in T-cell subsets characterized by an increase in T-suppressor/cytotoxic (CD8) cells and a reduction in the relative numbers of T-helper/inducer cell (CD4) and in the CD4/CD8 ratio was also reported in the high-dose treated group compared to the control. No effects were noted on total lymphocytes or B-cells indicating that T-cell subsets were preferentially affected by PCBs. However, no treatment-related effects were noted on the T-lymphocyte subsets when the analysis was repeated at 55 months of exposure (*19*). The absence of detectable effects on the lymphocyte subpopulations at 55 months into the study is indicative of the degree of adaptability of the immune system and emphasizes the need for repeated measures of the lymphocyte subsets as the study progresses.

Likewise, a trend towards decreased levels of ^{3}H-thymidine incorporation by mitogen-induced lymphocyte proliferation in treated groups were noted only for PHA-P and ConA but not for the PWM. In contrast, proliferation to alloantigens (one-way mixed lymphocyte culture) did not prove to be a sensitive indicator for potential effects of Aroclor 1254 on T-cell function. Repeated measurements of the total serum immunoglobulin levels (IgG, IgM, and IgA) and serum protein fractions also did not prove to be sensitive parameters for effects of low levels of PCBs. A significant increase of NK cell activity was noted in the group of monkeys administered the 80 µg/kg doses and only at the effector:target cell ratio of 75:1. To determine whether interferon, a known modulator of NK activity (*71*), played a role in augmenting NK activity, levels of interferon in ConA-stimulated peripheral blood leukocytes were measured. Results were inconclusive: a biphasic response was obtained in which levels were increased for the 20- and 80-µg/kg bw dose groups but decreased for the 40-µg/kg bw dose group. In contrast, serum levels of thymosin alpha-1, a thymus-derived hormone known to augment immunologic reactivity in immunosuppressed mice (*71*) and to synergize with interferon to augment NK activity in mice (*72*), were significantly increased in a dose-related fashion.

The mononuclear phagocytic lineage of cells is important in antigen recognition, processing, and presentation to T and B lymphocytes. In this study a trend towards decreasing phagocytic activity (zymosan) and phorbol myristate acetate activation of peripheral blood phagocytic cells (chemiluminescence assay) was noted across all groups (5.0- and 20.0-µg/kg bw groups were not tested). Similarly, the time to peak reading of phorbol myristate acetate activated phagocytic cells was increased in the 40- and 80-µg/kg bw groups compared to the control, indicating that Aroclor 1254 may have affected the functional integrity of the phagocytic cells. However, interleukin-1 and tumor necrosis factor levels were not affected substantially by PCB treatment.

A statistically significant dose–response increase in total serum complement activity (CH_{50}) was also observed at 55 months of exposure. These observations are in agreement with previous results which indicated that complement levels were increased in children exposed to TCDD (*73*) and in mice exposed to low levels of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (*74*). The mechanism by which Aroclor 1254 and TCDD modulate serum complement activity remains to be elucidated.

PCBs at high levels have been shown to affect the endocrine system of rats (*75*); however, serum levels of corticosteroids (hydrocortisone) measured throughout the monkey study were not affected by treatment (*76*). This clearly indicates that the observed PCB effects on several of the immune parameters resulted from a direct effect of Aroclor 1254 on the immune system.

Human Studies

Population studies by Humphrey (*12*) and Kreiss (*77*) indicated that for the general population, fish consumption is the major source of exposure to PCBs. Mean serum PCB levels of 73-ng/ml (range of 25 to 366 ng/ml) were reported for humans who consumed on the average 24 lb/year of fish from Lake Michigan (*12*). Also, the geometric mean for PCB levels in sera from a fish-eating population in Triana, Alabama, was 17.2 ng/ml (range of 3.2 – 158 ng/ml) with an arithmetic mean of 22.2 ng/ml (*77*). Excluding occupational exposure, these levels are much higher than the levels of PCB found in the general North American population, which are normally less than 10 ng/ml (*77*).

The effects of chemicals on the immune system of breast-fed infants whose mothers consumed contaminated Great Lakes fish have been examined in the maternal–infant cohort epidemiologic studies of Smith et al. (*78*) (the Wisconsin study) and Humphrey (*12*) (the Michigan study). The results of these epidemiologic studies summarized by Swain (*79*) indicated that the maternal serum PCB level during pregnancy was positively associated with the number and type of infectious illnesses suffered by the infant during the first 4 months of life and that the majority of infections were of bacterial origin. The incidence of infections of microbial etiology correlated strongly with the highest rate of fish consumption (at least 3 times per month for 3 years) and with the cumulative lifetime fish consumption levels.

Similar effects of PCBs on breast-feeding infants were also reported by Hara (*80*). These infants were born to women exposed to Kanechlor 500 (chlorine content, 55%) and Kanechlor 300 (chlorine content, 43%) while working in a capacitor factory in Shiga Prefecture, near Lake Biwa, Japan. The level of PCBs in breast milk were 30 to 200 ppb, which correlated strongly with blood PCB levels. A higher incidence of colds and gastrointestinal (vomiting, abdominal pain) and dermatological (eczema, itchy skin) manifestations were observed in breast-feeding infants born to the PCB-exposed women than in those infants born to nonexposed women. The incidence of these symptoms increased with increasing length of breast-feeding; however functional aspects of the immune system were not investigated in these children.

A recently completed human study by Svensson et al. (*81*) reported that consumption of fatty fish species, like salmon and herring, from the Baltic sea had a profound effect on the natural killer cell activity of these humans compared to a control nonfish eating population. However, the clinical significance of these findings is not clear because the presence and incidence of malignancies or infections were not reported. Furthermore, data on levels of PCBs in the blood or fat of the population studied, which would permit correlations between NK activity and levels of PCBs, were not included in this study.

Immune alterations of potential biological significance have been reported in the Japanese (Yusho) and Taiwanese

(Yu-Cheng) populations exposed to PCBs, PCDFs, and PCQs via the ingestion of contaminated rice oil (*82–87*). Kuratsune (*85*) estimated the total amount of rice oil contaminants consumed by the exposed (Yusho) population to be on the average 633 mg of PCB, 3.4 mg of PCDF, and 596 mg of PCQ, which corresponds roughly to 157, 0.9, and 148 µg/kg bw/day for PCB, PCDF, and PCQ, respectively. At this level of exposure, the length of the latent period between exposure and onset of clinical illness was approximately 71 days, with a wide range of 20 to 190 days. PCB levels were also determined in retrospective studies of the Yu-Cheng patients who consumed contaminated rice oil for 3 to 9 months. The PCB levels in the patients' blood collected at about 9 to 18 months after the onset of the disease were in the range from 10 to 720ppb with a mean value of 38 ppb (*87*).

The immunological investigations in these patients are summarized as follows. Persistent respiratory distress accompanied by Gram-negative bacilli-infected airways were observed in about half of the Yu-Cheng cases examined; however, there was no evidence of clinically defined bronchial asthma or pulmonary emphysema (*84*). A significant decrease in serum IgA and IgM but not IgG was noted at 2 years following the exposure, but levels returned to normal after 3 years (*85*). A statistically significant reduction in the percent of total T lymphocytes, apparently due to a reduction in T-helper cells and enhanced responses of peripheral blood leukocytes to nonspecific mitogens PHA, ConA, and PWM, was observed in the exposed Yu-Cheng individuals at 1 and 3 years after the onset of the disease (*85,86*). The Yusho patients tested at 14 years after exposure showed a slight increase in T-helper cells and a slight decrease in T-suppressor cells (*82,85*); statistically significantly reduced numbers of Yu-Cheng patients, compared to control, had positive skin test reactivity to streptokinase/streptodornase antigen mixture and to tuberculin antigens tested at 1 (streptokinase/streptodornase) and 4 years (tuberculin) after exposure (*83,86,87*). The percent of patients showing a skin test response and the size of the response decreased with increased severity of the clinically observed PCB-induced dermal lesions and also with PCB concentrations in the whole blood (*82,83*).

In contrast to the Yusho and Yu-Cheng populations, which were exposed to PCDF and PCQ in addition to PCBs, the study by Stark et al. (*88*) examined 52 individuals exposed only to PCBs (spill resulting from a transformer explosion in Syracuse, New York). Statistically significantly increased levels in the exposed group compared to controls (nonexposed individuals) were found for red blood cells, hemoglobin, hematocrit, and red blood cell mass but not for WBC.

Emmett et al. (*89,90*) compared 55 transformer repairmen (38 currently exposed and 17 previously exposed to PCBs) with 56 nonexposed subjects. PCB exposure, predominantly from Aroclor 1260 and to a lesser extent from exposure to Aroclor 1242, occurred from air and contaminated surfaces. Clinical manifestations typical of PCB poisoning were not present in these individuals. Likewise, the proportions of positive skin responses to mumps antigen (92% exposed, 89% control) and Trichophyton antigens (17% exposed, 8% control) did not differ significantly. The mean diameters of the skin reactions between the exposed and nonexposed subjects to mumps antigen in the two groups were identical (12.6 mm). Thus delayed hypersensitivity was not affected.

Lawton et al. (*91*) compared 194 capacitor manufacturing workers (152 males and 42 females) exposed occupationally to Aroclor 1016 with 41 other workers that had previously been exposed to Aroclors 1242 and/or 1254. These were examined in 1976 and in 1979, 2 years after discontinuance of PCB use in the operation. At the two examinations, the approximate geometric mean serum levels were 363 ppb (57–2270) for Aroclor 1016 and 68 ppb (12–393) for Aroclor 1242 and 1254, with 5 to 95% ranges of 30 (6–142) and 19 (4–108), respectively. In the study population, the mean service duration was 17 years (range 2–35) and the mean age was 40 years (range 20–65). Determinations in 1976 showed elevations in total WBCs associated with decreased polymorphonuclear cells and increased lymphocytes, monocytes, and eosinophils. In 1979 there were marginal increases in monocytes and eosinophils, but the WBCs were near normal. The statistical association of serum PCB levels and the monocyte counts was strong.

Chase et al. (*92*) investigated 120 male workers (86 exposed, 15 minimally exposed, and 19 nonexposed) in a railroad passenger car and locomotive maintenance facility where workers were exposed to PCB-containing transformer fluids for 40 years. The average age of the exposed and nonexposed populations was 41.4 and 30.7 years, respectively. The average plasma PCB levels were 33.4 ppb in the exposed group and 14.2 ppb in the unexposed group. No effect on serum albumin, globulin, or total protein was found.

Maroni (*93*) evaluated 80 electrical capacitor manufacture workers (40 female, 40 males) exposed to Piralene 3010 (a PCB mixture with 42% chlorine content) for a mean of 12 ± 6 years. The mean whole blood PCB concentration was approximately 450 µg/kg with a range of 310 to 495 µg/kg. Serum electrophoretic fractions and blood cell counts were not affected.

Smith et al. (*78*) examined three groups of workers occupationally exposed to Aroclor 1242 and Aroclor 1016. The three groups of workers were from an electrical equipment manufacturing plant, a public utility company, and a private utility company. The geometric mean of serum PCB concentrations among exposed workers was 8 to 50 times the community background levels, which ranged from 11.6 to 12.8 ng/ml (arithmetic mean). No effects were found on hematological parameters (hemoglobin, hematocrit, leukocyte, and differential counts), total serum protein, and serum albumin.

Finally, Stehr-Green et al. (*94*) examined environmentally exposed individuals. The levels of PCBs were 10.3 and 8.9 ppb in males and females, respectively. A direct relationship of age with serum PCB levels was evident. There were no investigations on hematological or other immunological parameters.

Discussion and Summary

The discipline of immunotoxicology is a relatively new concept and, unlike toxicity studies that are based on a set of well-defined toxicologic parameters detailed in the Organization for Economic Cooperation and Development guidelines, similar guidelines for immunotoxicity testing are only at the developmental stages (*27,95*). Furthermore, the concept of Good Laboratory Practices, which is applied to nonclinical toxicologic studies designed for regulatory purposes, is a relatively new concept to the immunotoxicologist. Consequently, there is an apparent lack of uniformity across the studies with regard to the animal model used, the choice of parameters examined, the number of doses used, and route and duration of exposure. These apparent study deficiencies and the use of high exposure levels, with the potential of producing toxicologic effects

which may have affected the immune system indirectly, diminish the usefulness of the derived data for regulatory purposes. However, such studies have generated valuable information regarding sensitive targets for immunotoxicity. This information can be used in designing multiple dose studies for immunologic end points and thus contribute to risk assessment.

Such studies demonstrated that commercially available PCB mixtures investigated alone affect several morphologic and functional aspects of the immune system in rodents, rabbits, guinea pigs, and chickens. The degree of severity of these effects varied across the studies and depended on the route and duration of exposure and the dose used, the PCB mixture studied, the species, and the age and sex of the animal. In general, such studies indicated that the higher chlorinated PCB mixtures might be more immunotoxic than the lower chlorinated mixtures.

Investigations on the effects of PCBs on serum protein fractions, including the percent serum gamma globulin fraction and the numbers of globulin-producing plasma cells in popliteal lymph nodes and in germinal centers of the spleen, have produced inconclusive results. Reduced leukocyte and T-lymphocyte levels in peripheral blood were reported following exposure to PCBs, while data regarding the effects of PCBs on total serum immunoglobulin levels have not been reported in nonimmunized animals.

No generalizations regarding effects on the thymus, spleen, and lymph nodes can be made across species. Decreases in thymus weight were observed in rats but not in guinea pigs or mice. Spleen weight and the microscopic structure of the spleen and thymus was not affected in rats, guinea pigs, or mice. In contrast, dermal and oral exposure to PCBs resulted in marked thymic atrophy characterized by loss of thymic cortical lymphocytes and reduction of germinal center size in the spleen of chickens and rabbits. Studies on the *in vitro* mitogen-induced responses of splenic mononuclear leukocytes indicated that PHA-induced leukocyte blastogenic activity was increased, while no effect was noted when ConA, STM, or PWM were used. This suggests that PCBs may affect a selected subpopulation of T lymphocytes. Measurements of the mixed lymphocyte responses, another *in vitro* correlate of cell-mediated immunity, remained unaffected by the lower chlorinated biphenyls while the GVH reaction was only transiently increased at high doses of PCB treatment.

In addition to humoral and cell-mediated immune effects, PCBs have been shown to affect the function of the mononuclear phagocytic lineage of cells. Functional impairment in PCB-exposed animals is characterized by reduced phagocytic activity and clearance of pathogenic bacteria by the spleen and liver, decreased resistance to viruses, and increased sensitivity to bacterial endotoxins.

Decreased natural killer cell activity has been reported for Aroclor 1254. However, despite the evidence that PCB-induced immunosuppression impairs immune surveillance, Aroclor 1254 was shown to protect mice and rats (shown as reduced tumor growth and metastasis) against certain kinds of experimentally induced tumors including the Ehrlich's tumor ascites and the primary Walker 256 tumor (*43,44*). This paradox points to the need for additional studies on PCB-induced carcinogenesis.

The existing limited data on the interactive effects of PCBs suggest that aromatic hydrocarbons that act through the Ah receptor (*96*) may have an additive effect when tested in mixtures, or they may antagonize the immunotoxic effects of other chemicals such as dioxins, which are also present in the Great Lakes. From the regulatory point of view, this concept is significant especially when the magnitude of effects of Great Lakes contaminants on human health is evaluated, because any effects on the immune system would be the resulting net effect of the interaction of all chemicals bioaccumulated in the tissues of fish.

The studies in which fish from the Great Lakes were used as the source of mixtures of contaminants indicated that these contaminants had an adverse immunomodulating effect at least in mice and rats. However, the difficulties encountered in defining an appropriate control group for studies of this kind ultimately influence the interpretation of results. For example, in the rat studies by Villeneuve et al. (*8*) and Chu et al. (*64*), the Pacific coho salmon was used as a control. This decision was based on the observation that the level of contaminants in this fish was much lower than that of the Lake Ontario coho salmon (*8*). However, effects were noted for both sources of salmon. Furthermore, a comparison of data from the treated groups to a control (rat chow) group may not be experimentally meaningful due to the existing qualitative differences in protein composition among the various diets. For example, the mouse study by Cleland et al. (*65*) used a control group of mice to which all other results were compared. As expected, statistically significant reduced immune reactivity was observed for the Lake Ontario coho salmon compared to the Pacific coho salmon. However, comparison of these data to the control group of mice may not be valid because there was no fish protein in the control diet.

The Great Lakes health effects rat study by Tryphonas et al. (manuscript in preparation) tested salmon from two sources—the Credit River, which runs into Lake Ontario, and Lake Huron in the region of Owen Sound. Previous residue analysis revealed that the Owen Sound fish contain contaminants qualitatively similar to those found in Lake Ontario fish but at reduced concentrations. Consequently, the Owen Sound fish were included in the study as a second control (low level of fish contaminants) group in addition to the rat chow control group. The rat chow control group consisted of rats fed rat chow supplemented with casein to a level equal to that of the fish-containing diets.

Preliminary statistical analysis of available data indicated that the direction of the observed effects on the immune system was not always consistent with the presumed differences in the level of contaminants in the fish diets. This suggests that factors such as qualitative and quantitative differences in fish contaminants and the potential immunomodulating effects of fish oils might be influencing the outcome of effects. Data on the qualitative and quantitative differences in residues found in the tissues of rats administered the fish diets will undoubtedly aid in the interpretation of the observed results. Ideally, one should use a control group exposed to fish diets derived from a noncontaminated environment. However, such a source of fish is not easily found.

The use of nonhuman primates to study the effects of PCBs on the immune system is desirable due to the phylogenetic and biologic similarities of monkeys to humans. This allows for extrapolation of results to the human situation to be made with a greater degree of confidence than when such extrapolation is made from data derived using other animal models. A common finding in all studies employing nonhuman primates is that immunization with SRBC antigens results in reduced antibody titers in PCB exposed monkeys compared to controls. This is true also for the chronic study carried out in rhesus monkeys whereby low levels of PCBs (Aroclor 1254)

were employed. In this study, a statistically significantly reduced antibody titer to SRBC was observed at PCB dose levels as low as 0.005 mg/kg bw/day. A no adverse effect level was not identified in this study. However, the potential interactive effects of Aroclors or mixtures of PCBs and other contaminants, which are readily detectable in the Great Lakes fish, need to be investigated before a holistic evaluation of the risk that these chemicals pose to human health can be made.

Ideally, any assessment of the magnitude of the risk that PCBs pose to human health should be supported by data derived from studies involving humans exposed to PCBs either through their occupation or through the ingestion of contaminated food, primarily fish. However, the strengths and advantages to the use of human data are often outweighed by the inherent study design weaknesses, often contradictory results among the different studies, and the methodological difficulties in determining actual exposure doses and in establishing reliable and reproducible dose–response relationships. Other confounding factors such as steroids and antibiotics may be important in epidemiologic studies in which the effect of PCBs on mother–infant cohorts are investigated. It is known that human breast milk, but not cow's milk, contains a steroid that inhibits glucuronyltransferase activity required for glucuronidation and excretion of PCB metabolites (*97*). Also certain antibiotics, including novobiocin, are known to noncompetitively inhibit glucuronyltransferase activity *in vitro* (*97*) thus increasing the body's burden of PCBs and the degree of risk posed to these infants and children. All these factors need to be considered in the design of epidemiologic studies and more importantly in the interpretation of results derived from such studies. While these data limitations present an obstacle to the establishment of a potential link between exposure to PCBs and adverse immune effects, the existing human studies provide valuable guidance for the design of additional perspective studies of populations exposed to PCBs via the consumption of Great Lakes-derived fish. Determination of PCB concentrations in blood and fat tissues from humans without occupational exposure indicates that background levels average approximately 10 ppb for blood and 1 ppm for adipose tissue (*96*). These levels parallel the levels of PCBs (Aroclor 1254) measured in blood and fat of the rhesus monkeys exposed to 5 µg/kg bw/day of Aroclor 1254 in the Tryphonas et al. studies (*18–20*) and for which immune suppression was detected. However, with the exception of the Yusho and Yu-Cheng studies for which limited immunological investigations were included in follow-up studies, functional parameters of the immune system were not investigated in any of the occupationally exposed cohorts. Lack of such data necessitates that extrapolation of results derived from the monkey studies to humans be made with caution. The observed increased incidence of bacterial infections in the mother–infant studies of fish-eating cohorts is of interest and suggests that the B cell might be affected. Yet the studies in monkeys and in other experimental animals in which the antibody response to various exogenous antigens was reduced suggested that the effect of PCBs might be at the antigen-presenting cell level (mononuclear phagocytic lineage of cells) or at the T-cell level. The changes observed in the T lymphocyte and their subsets in the Yusho (*84*) and Yu-Cheng (*84*) studies provide additional support for this hypothesis.

Future Considerations

Additional studies are required to define appropriate experimental models in which sensitive immunologic end points with biological relevance to the human situation can be identified; to improve our understanding of the mechanism(s) that might be operable in PCB-induced immunomodulation; and to standardize experimental designs, including the choice of immunological assays used in PCB immunotoxicity studies. Parallel to these requirements, there is a need to ensure that the principles of good laboratory practice be implemented and adhered to during the course of immunotoxicity studies.

Further research is required to better define the potential interaction among PCB mixtures or among mixtures of PCBs and the many other chemicals that accumulate in fish tissues. This will assist the regulator in better defining the net immunotoxic effect produced by these chemicals.

The effects of individual PCB congeners and of mixtures of PCBs on quantitative and functional aspects of the immune system of experimentally treated laboratory animals and studies on humans exposed to PCBs, either through their occupation or through ingestion of contaminated food, are an important component of risk assessment methodology. Few such studies are available. Regarding the effects of PCBs on human health, attention should be focused on those subpopulations who are especially vulnerable to such effects. These subpopulations would include people whose immune systems are already immunosuppressed either through medication or due to certain disease states, as well as infants whose immune systems are still developing and the aged whose immune systems are becoming less effective.

Future immunotoxicity studies are required for those PCB congeners that tend to persist in the environment for a long time and which are found in the blood and tissues of highly exposed population groups.

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