TOXICOLOGICAL SCIENCES 41, 49–61 (1998)
ARTICLE NO. TX972386

Symposium Overview: Toxicity of Non-Coplanar PCBs[1,2]

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Received September 22, 1997

Toxicity of Non-Coplanar PCBs. Fischer, L. J., Seegal, R. F., Ganey, P. E., Pessah, I. N., and Kodavanti, P. R. S. (1998). *Toxicol. Sci.* 41, 49–61.

Research into the mechanism of toxicity of PCBs has focused on the Ah receptor. However, it is becoming increasingly clear that certain ortho-chlorine-substituted, non-coplanar PCB congeners having low affinity for the Ah receptor exhibit important biological activities. Actions of non-coplanar PCB congeners in a variety of biological systems have been discovered and the mechanisms for these effects are being elucidated. The objectives of this symposium are to examine the state of knowledge concerning the mechanisms of toxic action of non-coplanar PCBs and to identify similarities and differences using a variety of biological systems. Effects to be considered will include: neurotoxicity, estrogenicity, insulin release, neutrophil function, calcium regulation, and relevant signal transduction systems. Finally, the symposium addresses the need to consider non-coplanar congeners within the context of risk assessment. The use of Ah-receptor binding and its associated biological effects to assess the total toxicity of PCBs may no longer be defensible because of the actions produced by non-coplanar congeners. This symposium provides documentation for that conclusion and focuses attention on emerging mechanisms of PCB action that have received relatively little attention to date. The topics presented should be of interest to toxicologists interested in mechanisms of action, in PCB risk assessment, and in regulatory toxicology.

Polychlorinated biphenyls (PCBs) are ubiquitous, persistent environmental contaminants resulting from intensive industrial use and inadequate disposal over past decades. They were used as commercial mixtures (e.g., Aroclors) containing up to 209 possible congeners (DeVoogt *et al.*, 1989; Mullin *et al.*, 1984). Their lipophilic character, particularly of the more highly chlorinated congeners in the mixture, permits concentration in the food chain and exposure to humans and wildlife. Mixtures and individual congeners possess a surprising array of biological activity leading to toxicity in laboratory animals. Human exposure to PCBs has been associated with skin disorders and possibly cancer, immune dysfunction, behavioral changes, and reproductive/developmental abnormalities (for a review see Swanson *et al.*, 1995).

PCB congeners that can assume a coplanar configuration are capable of binding the aryl hydrocarbon receptor (Ah receptor) and in so doing initiate biological actions and toxicity similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a chlorinated polyaromatic hydrocarbon known to bind to the receptor (Safe, 1994). In contrast, congeners possessing chlorine substitution at the *ortho* positions of the biphenyl rings are non-coplanar and do not bind with high affinity to the Ah receptor (Kafafi *et al.*, 1993). Originally thought to be biologically inactive, it is now known that non-coplanar congeners can exhibit toxic effects.

Emerging evidence suggests that certain ortho-substituted PCB congeners are partially responsible for the neurotoxic effects of PCBs, including decreased catecholamine levels in certain brain regions and behavioral changes in laboratory animals (Shain *et al.*, 1991; Seegal *et al.*, 1990, 1991b,c; Schantz *et al.*, 1995). Behavioral changes and learning deficits have been observed in monkeys (Schantz, 1997), rats (Pantaleoni *et al.*, 1988), and humans exposed perinatally to environmental PCBs (Jacobson *et al.*, 1990; Jacobson and Jacobson, 1996; Rogan and Gladen, 1992; Chen and Hsu, 1994; Chen *et al.*, 1994; Huisman *et al.*, 1995). It is also known that PCBs present in the environment are slowly being biotransformed, resulting in a higher proportion of non-coplanar congeners present in environmental samples than originally present in commercial mixtures. The PCBs found in blood and other tissues of humans, wildlife, and

[1] Symposium held at the 36th Annual Meeting of the Society of Toxicology, Cincinnati, OH.

[2] The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Drs. Hugh Tilson and Jim McKinney are acknowledged for their critical review on an earlier version of the manuscript.

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1096-6080/98 $25.00

fish exposed via the food chain to environmental PCBs are also primarily non-coplanar congeners (Patterson *et al.*, 1994; Johansen *et al.*, 1996).

The potential for toxicity from exposures to non-coplanar PCBs has been demonstrated, and information regarding the mechanisms of action of these compounds is now becoming available. Knowledge of biochemical mechanisms of action for non-coplanar congeners is required for meaningful risk assessments connected with low-level exposures to humans and wildlife. The following presentations describe experimental results that represent newer information regarding the mechanisms involved in the action of non-coplanar PCBs in different biological systems.

INSULIN RELEASE PRODUCED BY NON-COPLANAR PCBs (L. J. Fischer and M. A. Wagner)

Insulin release is stimulated from pancreatic β-cells in the islets of Langerhans by glucose, certain amino acids, and sulfonylurea drugs via a mechanism that involves an increase in intracellular free calcium (Hellman *et al.*, 1992). Published reports indicate that an alteration of calcium homeostasis in cells by non-coplanar PCBs and commercial mixtures of PCBs produces functional changes in cerebellar granular cells (Kodavanti *et al.*, 1993a), mammalian brain (Wong *et al.*, 1997a), and neutrophils (Brown and Ganey, 1995). This information prompted an investigation of the action of PCBs to alter synthesis and secretion of insulin from cells producing the hormone (Fischer *et al.*, 1996). RINm5F cells, derived from a rat insulinoma, have been used successfully as a model system to examine mechanisms of insulin secretion and synthesis and to examine the ability of xenobiotic chemicals to alter the function of pancreatic β-cells (Li *et al.*, 1990; Yada *et al.*, 1989). At the present time, this laboratory is investigating the mechanisms by which PCBs produce a stimulation of insulin release from RINm5F cells as part of an overall effort to examine the effects of environmental chemicals on the function of the endocrine pancreas.

Exposure of RINm5F cells in culture to Aroclor 1254 showed a concentration-dependent decline in cellular insulin levels after 2 days of exposure (Fischer *et al.*, 1996). A 40% decline was observed with 10 μg/ml of the Aroclor in the culture media but no loss occurred using 5 μg/ml. A 6-day exposure (unpublished results) showed a 40% loss of cellular insulin at 1.0 μg/ml of the Aroclor and an 85% reduction with 5 μg/ml of the PCB mixture. Media insulin levels during a 2- or 6-day exposure were less responsive to the presence of the Aroclor. No change was observed after 2 days and a 20% increase in media insulin occurred after 6 days of exposure. No PCB-related cytotoxicity was observed in the cultured cells during the exposure periods.

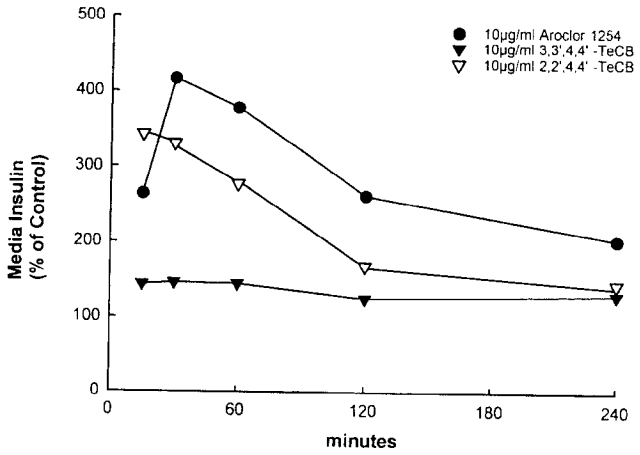

FIG. 1. Comparison of the time-course of insulin release into media produced by 10 μg/ml of Aroclor-1254, the non-coplanar congener 2,2',4,4'-TeCB, and the coplanar congener 3,3',4,4'-TeCB (data from Fischer *et al.*, 1996).

Whereas there were minimal apparent effects of Aroclor 1254 on insulin released to media in longer-term experiments, short-term exposure of cells to 5 and 10 μg/ml produced a concentration-dependent release of insulin into media (Fischer *et al.*, 1996). This effect was rapid and somewhat transient. It was observed after 15 min of exposure and the rate of release slowed after the first hour (Fig. 1). The cells became refractory to the effect because after a 2-h exposure and a 30-min washout period, the stimulatory action of PCBs was greatly reduced upon reexposure of the cells to Aroclor 1254 at the same concentrations (data not shown). A 37% loss of cellular insulin can be observed after 2 h of treatment with 10 μg/ml of the Aroclor, whereas no loss of cellular insulin occurs during this period using 5 μg/ml.

A decline in cellular insulin upon Aroclor 1254 exposure is apparently due to an insulin releasing action of the chemical mixture and also a deficiency in insulin biosynthesis. A concentration-dependent inhibition of [^{3}H]leucine incorporation into proinsulin and insulin was observed in RINm5F cells over a 24-h period (Fischer *et al.*, 1996). This effect may be due to a nonspecific action on protein synthesis because total protein synthesis in exposed cells was found to be inhibited to the same extent as proinsulin/insulin synthesis. The effect resulted in a 30% inhibition in cells treated with 10 μg/ml Aroclor 1254 relative to levels of incorporation in untreated control cells.

Recent experiments have been directed toward characterizing the mechanistic aspects of the insulin-releasing action of PCBs in RINm5F cells. Coplanar PCBs are known to bind to the Ah receptor as the initial event in producing their biological actions while non-coplanar PCBs initiate actions

by mechanisms that remain unclear as these compounds do not bind, or bind poorly, to the Ah receptor. The insulin-releasing activity of non-coplanar and coplanar congeners when assessed in the same experiment indicates that the latter type are relatively inactive compared to the rapid insulin release provided by non-coplanar congeners (Fischer *et al.*, 1996). For example, 3,3′,4,4′-tetrachlorobiphenyl (TeCB) is relatively inactive compared to the insulin release produced by 2,2′,4,4′-TeCB and the Aroclor 1254 mixture as shown in Fig. 1. The environmentally prevalent non-coplanar congener 2,2′4,4′,5,5′-hexachlolobiphenyl (HCB) causes a release of insulin with a potency similar to or greater than that of Aroclor 1254 (not shown).

Similar to other reports from experiments in which the action on non-coplanar PCBs was evaluated in different biological systems, it was found that lower chlorinated congeners such as 2,2′-dichlorobiphenyl (DCB), 3,3′-DCB, and 2,3′,4-trichlorobiphenyl (TCB) have insulin-releasing activity comparable to that of 2,2′4,4′-TeCB. In summary, initial structure–activity investigations employing insulin cell function have yielded results consistent with those found in other biological systems (Shain *et al.*, 1991; Kodavanti *et al.*, 1995, 1996a). Non-coplanar congeners having four or more chlorine atoms exhibit insulin-releasing activity, whereas the corresponding coplanar analogs do not possess activity. Lower chlorinated congeners containing two or three chlorines appear to be as active as higher chlorinated congeners. However, there is incomplete information regarding a more precise assessment of the relationship between degree of non-coplanarity and biological activity for lightly chlorinated congeners.

Current experiments utilizing Indo-1 fluorescence indicate that a rise in intracellular free Ca^{2+} occurs within 30 s after applying 10 μg/ml of Aroclor 1254 to RINm5F cells (unpublished results). A similar effect occurs after exposing cells to an insulin-releasing non-coplanar congener, 2,2′,4,4′,5,5′-HCB. Withdrawal of Ca^{2+} from extracellular media or addition of the calcium channel blocker verapamil to Ca-containing media inhibits at least 70% of the insulin release caused by Aroclors or individual non-coplanar congeners. These initial results indicate that a major fraction of the insulin release caused by PCBs can be related to the opening of calcium channels, probably of the L-type, allowing a rise in free intracellular Ca^{2+}. At the present time, further experimentation is occurring in an attempt to implicate other signalling pathways in PCB-induced insulin release.

Results available to date indicate that non-coplanar PCB congeners and commercial mixtures of PCBs have the ability to release insulin from a model cell system. The toxicologic importance of this activity will not be known until results of insulin release experiments are obtained using primary insulin-producing cells and intact animals.

Acknowledgment. This work was supported by NIEHS/NIH Grant ES04911.

NEUROCHEMICAL AND NEUROENDOCRINE EFFECTS OF NON-COPLANAR AND COPLANAR PCBs

(R. F. Seegal, J. F. Gierthy, K. F. Arcaro, and K. O. Brosch)

Studies of the neurobehavioral and neurotoxicological effects of PCBs in intact animals are hampered by the large number of potentially active PCB congeners involved in environmental exposures and by the metabolism of certain PCB congeners that result in the generation of metabolites, including biphenylols, that may be more biologically active than the parent congener.

In vitro studies of neurotoxicity utilize techniques in which: (i) the ability to metabolize PCBs either is absent or occurs at a low rate and (ii) the neuronal preparations are considerably less complex than the intact brain. Hence the ability to extrapolate these findings to the whole animal may be limited. For example, Seegal *et al.* (1997) recently demonstrated that developmental exposure of the laboratory rodent to the coplanar congener 3,3′,4,4′-TCB significantly elevated regional brain DA concentrations. These findings represent the first example that PCBs increase, rather than decrease, DA concentrations, which has been shown in earlier studies from this laboratory (Seegal *et al.*, 1990). Thus a coplanar congener, shown to be inactive in both PC12 (Shain *et al.*, 1991) and cerebellar granule cells (Kodavanti *et al.*, 1995, 1996a), alters neurochemical function when presented during development. The mechanisms responsible for these unexpected elevations in brain DA in intact animals are not fully understood but may involve, in addition to binding to the Ah receptor, complex interactions between the estrogen-like activity of certain PCB congeners or their metabolites and regulation of DA function.

To experimentally address this hypothesis, Seegal, Gierthy, and colleagues have undertaken a series of studies designed to assess the estrogen-like activity of selected PCB congeners and their metabolites using both *in vitro* (MCF-7 human breast cancer cells in culture) and *in vivo* (rat prepubertal uterine wet weight assay) procedures.

MCF-7 cells were exposed for 13 days to various concentrations of 17β-estradiol, the tetra-*ortho*-substituted congener 2,2′,6,6′-TeCB, the coplanar congener 3,3′,4,4′-TeCB, and its hydroxy metabolite (i.e., 3,3′,4′,5-tetrachloro-4-biphenylol). The number of induced foci was measured using an automated colony counter modified to magnify the image of the multicellular foci.

Results presented in Fig. 2A clearly demonstrate two major points. First, the *ortho*-substituted PCB congener 2,2′,6,6′-TeCB, the coplanar PCB congener 3,3′,4,4′-TeCB, and its hydroxy metabolite induce significantly increased MCF-7 foci formation (with respect to vehicle-exposed

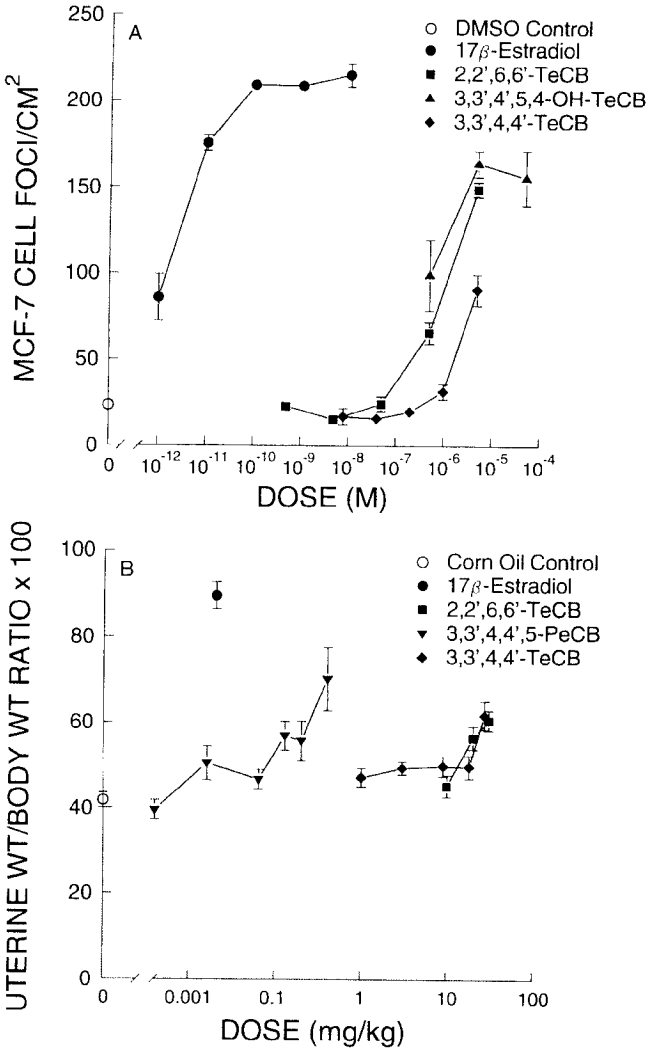

FIG. 2. (A) Effect of 13-day exposure of MCF-7 human breast cancer cells in culture to various concentrations of 17β-estradiol, 2,2',6,6'-TeCB, 3,3',4',5,4-OH-TeCB, or 3,3',4,4'-TeCB on foci formation (foci/cm^2) in postconfluent cells. (B) Effect of ip exposure of prepubertal female rats on days 21 and 22 to 20 μg/kg 17β-estradiol, or various concentrations of 2,2',6,6'-TeCB, 3,3',4,4',5-PeCB, or 3,3',4,4'-TeCB on uterine wet weight expressed as a ratio of uterine wet weight/body weight × 100.

cells) with EC50s ranging from 5×10^{-6} to 5×10^{-7}. Second, 17β-estradiol, with an EC50 of 5×10^{-11}, is much more active in inducing foci than any of the PCB congeners or their metabolites.

The above results obtained in tissue culture have been, to a large extent, replicated in the whole animal. For the prepubertal uterine weight assay Sprague–Dawley-derived female rats were injected intraperitoneally (ip) on postnatal days 21 and 22 with corn oil, 17β-estradiol (20 μg/kg), or various concentrations of 2,2',6,6'-TeCB, 3,3',4,4'-TeCB, or 3,3',4,4',5-PeCB and euthanized 24 h after the last injection. The uteri were removed, trimmed of connective tissue, and immediately weighed. All dissections were conducted in a blinded fashion and results from a minimum of four to six animals at each concentration are presented as a ratio of uterine weight/body weight × 100.

As seen in Fig. 2B, two ip injections of either the tetra-*ortho*-substituted congener 2,2',6,6'-TeCB or the coplanar congener 3,3',4,4'-TeCB significantly elevate uterine wet weights—results similar to those seen in MCF-7 cells (i.e., they exhibit weak estrogen-like activity). However, in contrast to the anti-estrogenic activity of the coplanar congener 3,3',4,4',5-PeCB measured in MCF-7 cells (data not presented), ip exposure of the prepubertal rat to this congener results in significant uterotropic activity. This disparity between the *in vitro* and *in vivo* results emphasizes the importance, and indeed the necessity, of carrying out parallel experiments using both biological systems to determine the estrogenic activity of PCB congeners and their metabolites.

The above results clearly demonstrate that certain coplanar and non-coplanar PCB congeners (or more likely their hydroxy metabolites) are weakly estrogenic. In support of these findings Nesaretnam *et al.* (1996) have recently demonstrated that 3,3',4,4'-TeCB (or one of its metabolites) binds to the human estrogen receptor, induces foci growth in MCF-7 cells, and is uterotropic. Further consideration raises the question: can the estrogen-like activity of these compounds, and in particular the activity of the coplanar PCB congener 3,3',4,4'-TeCB, be linked to nonreproductive changes in CNS function, including deficits in learning mediated by the prefrontal cortex?

Seegal *et al.* (1997) hypothesized that the elevations in regional brain DA concentrations following developmental exposure to 3,3',4,4'-TeCB may be due to the estrogen-like activity of its major hydroxy metabolite. Initial evidence to support this are reports that demonstrate the accumulation of the parent PCB congener and/or its metabolites in brain. Thus, within 72 h following an intravenous bolus dose of [^{14}C]3,3',4,4'-TeCB, this congener and/or its metabolites accumulates in the vascular space of prepubertal rat brain (Ness *et al.*, 1994). Similarly, following gestational exposure, Morse *et al.* (1995) have shown that 3,3',4',5-tetrachloro-4-biphenylol accumulates to high levels in the brains of fetuses and newborn rats.

More recent evidence gathered in this laboratory and others (e.g., Nesaretnam *et al.*, 1996) demonstrates that either the parent PCB congener or, more likely, its biphenylol metabolite is estrogen-like. Taken together, the presence of the biphenylol metabolite, combined with its estrogen-like activity, suggests that alterations in steroid hormone homeostasis, due to either contaminant-induced changes in steroid hormone metabolism or the endocrine-like actions of these contaminants, particularly during development, can lead to long-

term, if not permanent, changes in DA function that have been demonstrated previously using estradiol. These changes include increases in DA receptor number, brain DA concentrations, and tyrosine hydroxylase activity (Levesque and DiPaolo, 1989; Pasqualini *et al.*, 1995). In turn, alterations in brain DA concentrations (either increases or decreases), particularly in the prefrontal cortex, may result in behavioral deficits (Brozoski *et al.*, 1979; Sawaguchi *et al.*, 1989; Murphy *et al.*, 1996). Further strengthening the link between coplanar PCB and/or metabolite-induced changes in neurochemical function and behavior, Weinand-Harer *et al.* (1997) exposed rats from gestational day 10 to 18 to 1 mg/kg of 3,3′4,4′-TeCB (Seegal *et al.*, 1997) and noted behavioral deficits in passive avoidance and measures of drug-induced catalepsy.

In summary, the above data demonstrate that either parent PCB congeners or their metabolites exhibit estrogen-like activity and support the hypothesis that developmental exposure to a coplanar as well as a non-coplanar congener and/or their estrogenic metabolites can induce subtle, but long-term, changes in CNS function and behavior. This emphasizes the need to consider nonreproductive CNS changes in an assessment of the endocrine-disrupting actions of environmental contaminants, including PCBs.

Acknowledgment. Research support for this work was provided in part by grants under NIEHS ES04913 to R.F.S. and J.F.G.

MECHANISMS OF ACTIVATION OF NEUTROPHILS BY POLYCHLORINATED BIPHENYLS

(P. E. Ganey, P. K. Tithof, and A. P. Brown)

Neutrophils (polymorphonucleated leukocytes) serve a beneficial role in host defense by aiding in destruction of pathogens. A critical event in performance of this role is activation of these cells, which engages specialized functions such as production of reactive oxygen species (e.g., superoxide anion (O_2^-)) and secretion of proteases and cytokines. Thus, impairment of activation of neutrophils can lead to increased host susceptibility to the adverse effects of pathogens. On the other hand, unregulated or untimely activation of neutrophils and expression of these specialized functions can contribute to tissue injury (e.g., Ward *et al.*, 1983; Dahm *et al.*, 1991).

Neutrophils can be activated not only by endogenous inflammatory mediators but also by xenobiotic agents. Our early studies indicated that exposure of rat or human neutrophils *in vitro* to the PCB mixture Aroclor 1242 activated neutrophils to produce O_2^- and to undergo secretion of lysosomal products (Ganey *et al.*, 1993). These effects were concentration-dependent, occurring between 1 and 10 μg/ml Aroclor 1242. Furthermore, Aroclor 1242 increased generation of O_2^- in response to phorbol ester but decreased lysosomal secretion in neutrophils stimulated by the peptide *N*-formyl-methionyl-leucyl-phenylalanine (Ganey *et al.*, 1993). Interestingly, the effects of the PCB mixture could be mimicked by non-coplanar congeners with low affinity for the Ah receptor (e.g., 2,2′,4,4′-TeCB, 2,3,4,5-TeCB) but not by coplanar PCBs which bind the receptor with high affinity (e.g., 3,3′,4,4′-TeCB, 3,3′,4,4′,5-PeCB), suggesting that the primary mechanism of activation is independent of the Ah receptor (Ganey *et al.*, 1993; Brown and Ganey, 1995; Tithof *et al.*, 1995). These results with non-coplanar PCBs are consistent with those observed in other systems, including some discussed in this report (Shain *et al.*, 1991; Kodavanti *et al.*, 1995; Wong and Pessah, 1996).

Much is known about signal transduction pathways leading to activation of neutrophils, and studies were undertaken to determine which of these pathways are important in PCB-induced stimulation of neutrophils. Initial studies focused on phospholipase C-dependent hydrolysis of phosphatidylinositol-4,5-bisphosphate and subsequent release of intracellular calcium (Ca^{2+}). Aroclor 1242 induced an early release of inositol-1,4,5-trisphosphate, a product of phospholipase C activity (Tithof *et al.*, 1995) (Fig. 3). As with O_2^- production, 2,2′,4,4′-TeCB and not 3,3′,4,4′-TeCB also activated phospholipase C in neutrophils. Unlike other stimuli which activate phospholipase C in neutrophils, activation of neutrophils by PCBs did not appear to involve GTP-binding proteins because neither pertussis toxin, cholera toxin, nor the nonhydrolyzable GTP analog, GDPβS, affected O_2^- production in response to PCBs (Tithof *et al.*, 1997). A critical role for Ca^{2+} in PCB-induced activation of neutrophils was demonstrated by studies in which omission of Ca^{2+} from the extracellular medium or pretreatment with an antagonist of the mobilization of intracellular Ca^{2+}, TMB-8, inhibited both O_2^- generation and secretion in neutrophils exposed to 2,2′,4,4′-TeCB or 2,3,4,5-TeCB (Brown and Ganey, 1995).

Activation of neutrophils by PCBs was accompanied by phosphorylation of tyrosine residues of proteins, suggesting activation of tyrosine kinases (Tithof *et al.*, 1997). Tyrosine phosphorylation was increased in neutrophils exposed to Aroclor 1242, 2,2′,4,4′-TeCB, or 3,3′,4,4′,5-PeCB. This result was unexpected for 3,3′,4,4′,5-PeCB because this congener did not cause production of O_2^- in neutrophils. However, the duration of phosphorylation was more transient (<5 min) for this congener than for Aroclor 1242 or for 2,2′,4,4′-TeCB (>5 min). The phosphorylation product(s) has not been identified. Genistein, an inhibitor of tyrosine kinases, but not its inactive, structural analog daidzein, diminished PCB-induced O_2^- production by 60%, suggesting that activation of tyrosine kinases plays a role in stimulation of neutrophils by PCBs.

Another signal transduction pathway important in stimulation of neutrophils by some agents is activation of phospholi-

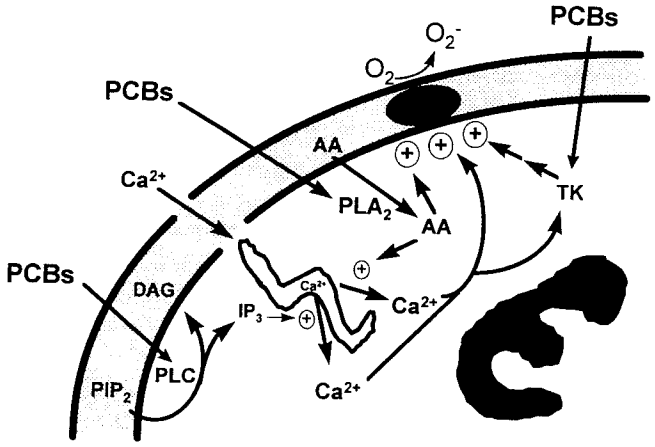

FIG. 3. Working hypothesis of signal transduction pathways involved in stimulation of neutrophils by PCBs. PCBs activate phospholipase C (PLC) leading to production of inositol-1,4,5-trisphosphate (IP_3) which can liberate Ca^{2+} from intracellular stores. Experimental evidence suggests that extracellular and intracellular Ca^{2+} are required for O_2^- production in response to PCBs. PCBs also activate phospholipase A_2 (PLA_2), leading to generation of arachidonic acid (AA), which contributes to activation of NADPH oxidase (Ox) that reduces O_2 to O_2^-. Inhibition of activity of PLA_2 reduces PCB-induced generation of O_2^-. Tyrosine kinase (TK) activity increases upon exposure of neutrophils to PCBs, and inhibition of this enzyme activity diminishes production of O_2^- in PCB-treated cells. It remains unknown whether activation of enzymes (i.e., PLC, PLA_2, or TKs) by PCBs occurs directly (as depicted) or is mediated by secondary events.

pase A_2 (PLA_2) and consequent release of arachidonic acid (Dana *et al.*, 1994; Henderson *et al.*, 1993) (Fig. 3). Arachidonic acid was released from neutrophils exposed to Aroclor 1242 or 2,2′,4,4′-TeCB, and release preceded the onset of O_2^- production (Tithof *et al.*, 1996). In addition, the concentration–response curves for arachidonic acid release and generation of O_2^- were similar. Of the two coplanar congeners examined, 3,3′,4,4′-TeCB did not cause release of arachidonic acid whereas 3,3′,4,4′,5-PeCB did. The reason for the activation of PLA_2 by 3,3′,4,4′,5-PeCB in the absence of O_2^- production is not known; however, previous studies with other agonists have demonstrated that arachidonic acid release is necessary, but not sufficient, for O_2^- generation in neutrophils (Henderson *et al.*, 1993; Ward *et al.*, 1985). The different functional responses may relate to activation of different isoforms of PLA_2 (Glaser, 1995). For example, the isoform of PLA_2 activated by the calcium ionophore A23187, which does not elicit O_2^- generation in neutrophils, is dependent on calcium and leads to metabolism of arachidonic acid to prostaglandins and leukotrienes (Tithof and Ganey, in press, *J. Immunol.*, 1988). Activation of PLA_2 by Aroclor 1242 occurred in the absence of extracellular Ca^{2+} and in the presence of the intracellular calcium chelator, BAPTA, suggesting that this isoform is calcium-independent. In support of this hypothesis, bromoenol lactone, which selectively inhibits calcium-independent isoforms of PLA_2 (Hazen *et al.*, 1991), reduced PCB-induced production of O_2^- in a concentration-dependent manner. In addition, more than 85% of the arachidonic acid released by neutrophils exposed to PCBs remained as parent compound, with little metabolism to eicosanoids. These results suggest that PCBs activate a calcium-independent PLA_2 which generates arachidonic acid as a second messenger and that this activation is important in generation of O_2^- by neutrophils.

In summary, three pathways have been identified which are important in activation of neutrophils by PCBs: release of intracellular Ca^{2+} (possibly through inositol-1,4,5-trisphosphate produced by activation of phospholipase C), tyrosine kinase activation, and arachidonic acid released by activation of PLA_2 (Fig. 3). It remains unknown whether activation of phospholipase C, PLA_2, or tyrosine kinase by PCBs occurs directly (as shown in Fig. 3) or is mediated by secondary events. Each pathway appears to be necessary, however, and interruption of any one dramatically reduces O_2^- production in response to non-coplanar PCB congeners. This suggests either that convergence of all three pathways is necessary for activation of neutrophils, or that these pathways occur in series. Elucidation of the sequence of events and the interactions among these pathways will likely shed light on the contribution of each of these possibilities and on the mechanism of activation of neutrophils by PCBs.

Acknowledgment. This work was supported by NIH/NIEHS ES04911.

AN IMMUNOPHILIN-MEDIATED MECHANISM FOR *ORTHO*-SUBSTITUTED POLYCHLORINATED BIPHENYL NEUROTOXICITY (I. N. Pessah and Patty W. Wong)

This laboratory has provided evidence for a stringent structure–activity relationship among PCBs possessing two or more chlorine substitutions in the *ortho* positions for activation of ryanodine-sensitive Ca^{2+} channels of mammalian striated muscle (skeletal and cardiac) and the central nervous system, revealing an Ah-receptor-independent mechanism through which PCBs disrupt Ca^{2+} signaling (Wong and Pessah, 1996; Wong *et al.*, 1997a). The most potent congener at the receptor yet identified, PCB 95 (2,2′3,5,6-pentachlorobiphenyl), was found to alter Ca^{2+} transport across neuronal microsomal membrane vesicles by a ryanodine receptor-mediated pathway, instead of an IP_3 receptor-mediated pathway. These actions of PCB 95 at ryanodine receptors may underlie its ability to alter neuronal excitability in the rat hippocampal slices *in vitro* (Wong *et al.*, 1997b) and both locomotor activity and spatial learning in an *in vivo* rat model (Schantz *et al.*, 1997). More generally, a ryanodine-receptor-mediated mechanism could account for the ability of non-coplanar PCBs to alter protein kinase C translocation and phosphoinositide metabolism in primary cerebellar granular cell cultures (Kodavanti *et al.*, 1993a, 1995).

Results from more detailed mechanistic studies have revealed that the actions of PCB 95 on microsomal Ca^{2+} transport and RyRs are mediated through the major T-cell immunophilin FKBP12 which is tightly associated with RyRs in muscle and brain (Wong and Pessah, 1997). The toxicological significance of immunophilin-mediated mechanisms by which *ortho*-substituted PCBs alter microsomal Ca^{2+} signaling and Ca^{2+}-dependent cascades may be far reaching. The level of high-affinity binding of [^{3}H]ryanodine (1 nM) to microsomal preparations was low when assayed in the presence of physiological concentration of monovalent cations. Incubation with 1 μM of the non-coplanar congener PCB 95 enhanced the specific occupancy of [^{3}H]ryanodine to RyR dose dependently (Fig. 4A). Although FK 506 did not significantly alter the high-affinity binding of [^{3}H]ryanodine to RyR, FK 506 did eliminate PCB 95-enhanced [^{3}H]ryanodine occupancy with an IC50 of 40 μM. These results demonstrated that at concentrations known to dissociate FKBP12 from RyR, FK 506 eliminated PCB 95-enhanced binding of [^{3}H]ryanodine to RyR, suggesting an immunophilin-dependent mechanism. Ca^{2+} transport measurements revealed that addition of 1 μM PCB 95 induced a net Ca^{2+} efflux from actively loaded SR vesicles (Fig. 4B, trace a). FK 506 (50 μM) introduced approximately 3 min prior to addition of 1 μM PCB 95 completely eliminated the response to PCB 95 (Fig. 4B, trace b). FK 506 selectively eliminated PCB 95-induced Ca^{2+} release from SR since Ry_1Rs maintained responsiveness to caffeine and Ca^{2+}-induced Ca^{2+} release (Wong and Pessah, 1997).

Marks and co-workers (Brillantes *et al.*, 1994) have shown that the high-affinity interaction between the RyR oligomer and FKBP12 is essential for stabilizing the native full conductance gating behavior of the SR Ca^{2+} release channel, since RyR expressed heterologously in the absence of FKBP12 exhibits several channel subconductances when reconstituted in bilayer lipid membranes. Further support of the functional importance of the immunophilin in stabilizing the RyR channel complex comes from pharmacological studies with immunosuppressant FK 506 and its analogs. Studies from several laboratories (Marks, 1996) have revealed that FK 506 is sufficient to dissociate FKBP12 from RyR, although it is not clear whether complete dissociation of the immunophilin is achieved. In the present study, FK 506 completely eliminated PCB 95-induced Ca^{2+} release and PCB 95-enhanced binding of [^{3}H]ryanodine to RyR in the same concentration range required to dissociate FKBP12 from RyR, indicating a strong correlation between the activity of PCB 95 toward RyR and the integrity of the FKBP12/RyR_1 complex.

Recent studies with brominated macrocyclic bastadins isolated from the marine sponge *Ianthella basta* have indicated that bastadin 5 enhances SR loading capacity by modulating

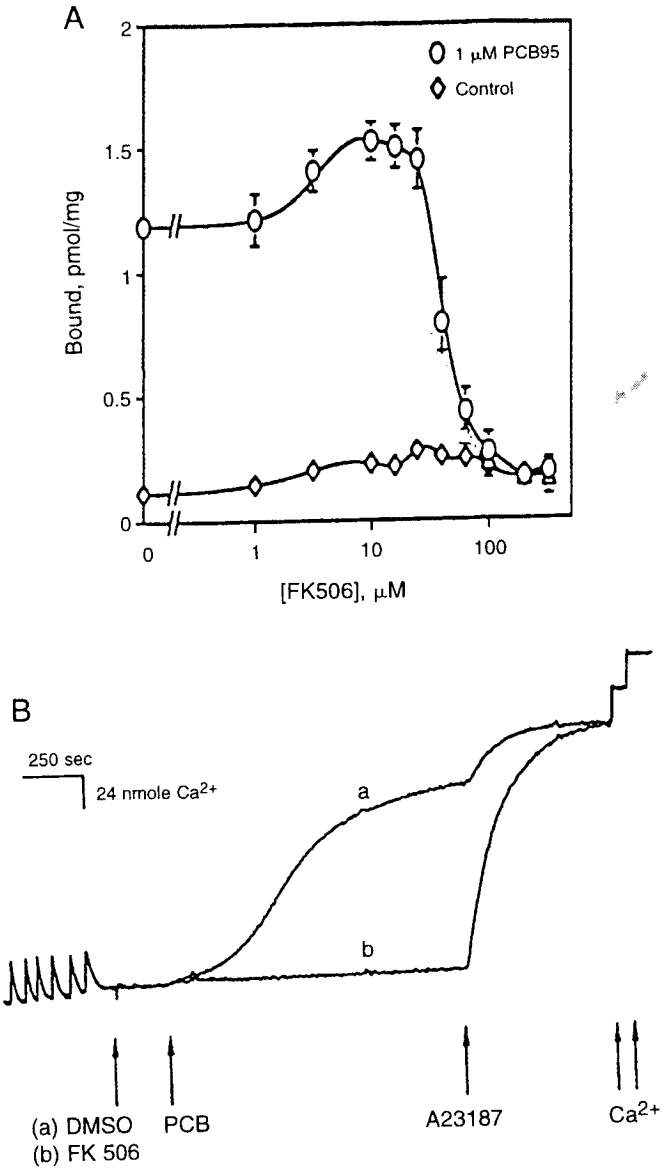

FIG. 4. Non-coplanar PCB 95 directly activates ryanodine-sensitive Ca^{2+} channels in isolated microsomal membranes in an immunophilin-dependent manner. In (A), the presence of PCB 95 enhances occupancy of [^{3}H]ryanodine to a conformationally sensitive site on the channel complex. Since high-affinity binding of [^{3}H]ryanodine to the channel occurs only when the channel is in an open state, the results are interpreted to mean that PCB 95 enhances channel activation. These actions of PCB 95 are wholly eliminated with the immunosuppressant FK506. FK506 is known to promote dissociation of the major T-cell immunophilin (FKBP12) from the ryanodine receptor channel complex. The results demonstrate that the Ca^{2+} modulating actions of PCB 95 may be mediated through an action on FKBP12. In (B), Ca^{2+} transport across isolated microsomes from skeletal muscle was measured directly. After active (ATP-driven) loading of Ca^{2+} into the membrane vesicles (with six bolus additions of Ca^{2+}, 20 nmol each), addition of 1 μM PCB 95 (trace a) rapidly releases the accumulated Ca^{2+}. However, if FK506 (50 μM) was introduced 3–5 min prior to PCB the actions of PCB 95 are fully eliminated (trace b).

the FKBP12/RyR complex and converting a ryanodine-insensitive efflux pathway ("leak") into a ryanodine-sensitive efflux pathway ("channel") which recognizes ryanodine with high affinity (Mack *et al.*, 1994). PCB 95 was shown to modulate [^{3}H]ryanodine binding sites in a manner very similar to that of bastadin 5 (Wong and Pessah, 1996; Wong *et al.*, 1997a). Both PCB 95 and bastadin 5 increased the affinity and capacity of high-affinity [^{3}H]ryanodine binding to RyR, and also significantly altered modulation of RyR by Ca^{2+} and Mg^{2+}.

To test the hypothesis that PCB 95, like bastadin 5, alters SR Ca^{2+} loading capacity by converting a ryanodine-insensitive Ca^{2+} efflux pathway (leak) normally present in SR into a ryanodine-sensitive efflux (channel), the SERCA pump inhibitor thapsigargin was employed. In the absence of ryanodine, addition of thapsigargin blocked SERCA pump activity, which would be expected to evoke Ca^{2+} efflux from actively loaded SR vesicles through both ryanodine-sensitive and -insensitive pathways. In contrast, pump blockade on actively loaded SR vesicles pretreated with high micromolar ryanodine should only unmask Ca^{2+} efflux through a ryanodine-insensitive pathway. Figure 5A (trace a) demonstrated that after completion of active Ca^{2+} loading under the control condition, addition of thapsigargin evoked release of accumulated Ca^{2+} even though extravesicular Ca^{2+} level was initially below threshold to activate calcium-induced calcium release (CICR). Pretreating vesicles with 500 μM ryanodine has been shown to completely block caffeine-induced Ca^{2+} release or CICR under conditions identical to those used here (Pessah *et al.*, 1997). Figure 5B (trace a) revealed that addition of thapsigargin after completion of Ca^{2+} loading to SR vesicles pretreated with 500 μM ryanodine unmasked a ryanodine-insensitive Ca^{2+} efflux pathway, consistent with previous findings (Pessah *et al.*, 1997). Coplanar PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl; 5 μM), a PCB congener lacking activity toward RyRs and SR/ER Ca^{2+} transport (Fig. 4), did not alter thapsigargin-evoked Ca^{2+} efflux regardless of whether the vesicles are pretreated with micromolar ryanodine (Figs. 5A and 5B, traces b). In marked contrast, 5 μM PCB 95 dramatically (386% of control, $p < 0.025$) enhanced the initial rate of Ca^{2+} efflux evoked by the addition of 375 nM thapsigargin (Fig. 5A, trace c). Importantly, in the presence of channel-blocking concentration of ryanodine, the ryanodine-insensitive component of Ca^{2+} efflux unmasked by addition of thapsigargin was greatly reduced by the presence of PCB 95 in a dose-dependent manner (Fig. 5B, trace c; Fig. 5C). The IC50 for the elimination of ryanodine-insensitive Ca^{2+} leak by PCB 95 was 3.5 $\pm$ 0.2 μM.

These results suggest that PCB 95, and other non-coplanar PCBs, through their actions on the FKBP12/RyR complex, relate ryanodine-insensitive and ryanodine-sensitive Ca^{2+} efflux pathways in native microsomes. Micromolar ryanodine eliminated CICR and caffeine-induced Ca^{2+} release, but not a major ryanodine-insensitive Ca^{2+} leak unmasked by SERCA pump blockade. In contrast, PCB 95: (1) enhanced net Ca^{2+} efflux from SR with the presence or absence of pump activity, and (2) eliminated the ryanodine-insensitive component of Ca^{2+} efflux (leak) unmasked by thapsigargin. The most direct interpretation of these results is that PCB 95 enhanced the proportion of channel to leak states of RyR on the SR membrane. Coplanar PCB 126 altered neither the ryanodine-sensitive Ca^{2+} efflux nor the ryanodine-insensitive Ca^{2+} leak unmasked by thapsigargin, demonstrating the structural specificity of the non-coplanar PCB 95 for eliciting the unique actions on microsomal Ca^{2+} transport. An immunophilin-mediated mechanism could explain the diverse biological activities attributed to *ortho*-substituted PCBs and may underlie toxicity.

NON-COPLANAR POLYCHLORINATED BIPHENYLS AND SECOND MESSENGER SYSTEMS IN NEURONS: STRUCTURE–ACTIVITY RELATIONSHIPS AND MODELS (Prasada Rao S. Kodavanti)

Several neurochemical endpoints are reported to be affected by PCBs, and these effects appeared to be related to the non-coplanarity of the PCB molecule. PCB-induced neurochemical effects include alterations in neurotransmitter levels, including dopamine (Seegal *et al.*, 1991a,b), inhibition of oxidative phosphorylation (Maier *et al.*, 1994), effects on neuroendocrine systems, including thyroid hormones (Porterfield, 1994), and perturbations in intracellular second messenger systems, including calcium disposition, inositol phosphates (IP), and protein kinase C (PKC) (for review, see Kodavanti and Tilson, 1997). Since intracellular second messengers play a vital role in neuronal growth and normal physiology of cells, research in this laboratory has focused on this possible cellular mechanism(s) of PCB neurotoxicity.

The distribution of Ca^{2+} within the cell is complex and involves binding to cell macromolecules and compartmentalization within the subcellular organelles (Farber, 1990). Normal physiological function of the cell is regulated by changes in intracellular free Ca^{2+} ($[Ca^{2+}]_i$), which ranges from 0.1 to 0.3 μM. This low level is maintained by the effective operation of Ca^{2+} pumps located in plasma membrane, endoplasmic reticulum (ER), and mitochondrion (Farber, 1990). Also, second messengers such as diacylglycerol (DAG) and inositol trisphosphate (IP_3) are generated by the activation of membrane receptor followed by phospholipid hydrolysis (Nishizuka, 1992). DAG activates PKC which, in turn, catalyzes the phosphorylation of a variety of cellular proteins. On the other hand, IP_3 releases Ca^{2+} from ER and regulates several processes including translocation and activation of

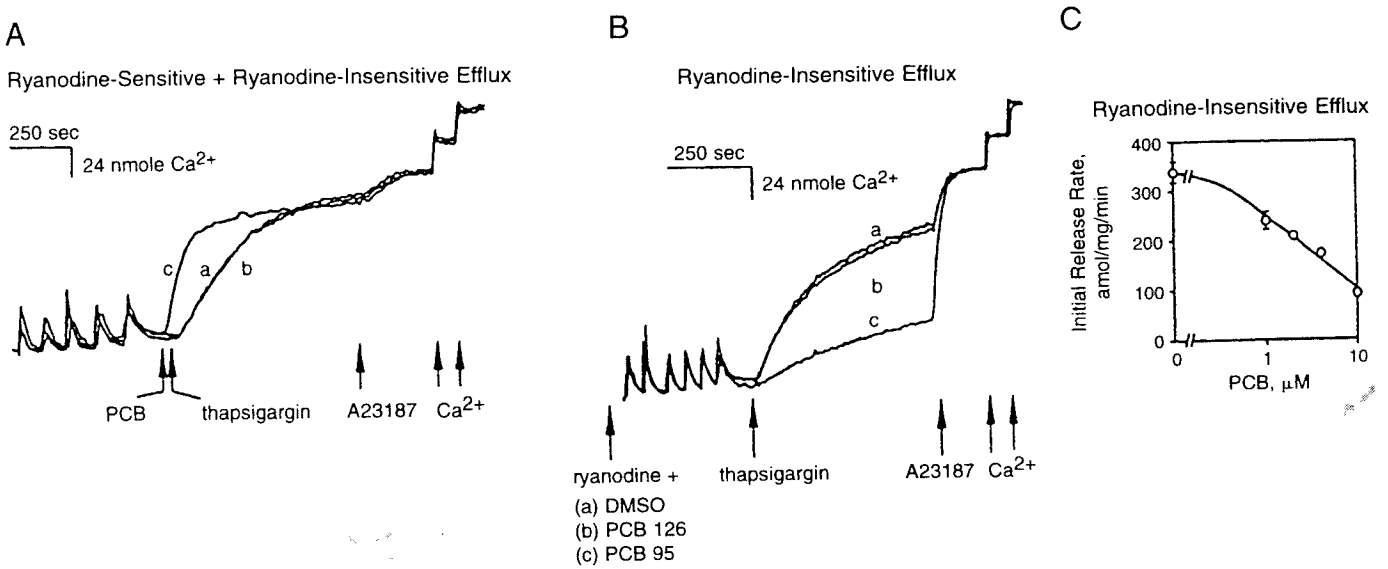

FIG. 5. Non-coplanar PCB 95 alters the Ca^{2+} filling capacity of microsomal vesicles by eliminating a ryanodine-insensitive Ca^{2+} leak pathway. Microsomal vesicles were actively loaded with Ca^{2+} as described in the legend to Fig. 4B. Trace (a) shows that SERCA pump inhibition with thapsigargin results in net efflux of accumulated Ca^{2+}. Trace (b) shows that microsomes preincubated with 500 μM ryanodine (to fully block ryanodine-sensitive Ca^{2+} channels) do not change the response to SERCA pump blockade. By contrast, PCB 95 (1 μM) dramatically increases the rate of Ca^{2+} efflux from the microsomes upon SERCA pump blockade (trace c). These results demonstrate the presence of ryanodine-sensitive and -insensitive Ca^{2+} release pathways in the microsomal membrane. In (B), microsomal membranes were pretreated with ryanodine (500 μM; to completely block sensitive channels) and DMSO (trace a), PCB 95 (trace b), or coplanar PCB 126 (trace c). The vesicles were actively loaded with Ca^{2+} and thapsigargin was added to determine the integrity of the ryanodine-insensitive Ca^{2+} leak pathway. Note that only the combination of ryanodine and PCB 95 significantly inhibits the Ca^{2+} leak pathway and this action is dose-dependent (C).

PKC (Fischer *et al.*, 1992). Long-term potentiation (LTP) is a form of synaptic plasticity and is often used as a physiological mechanism for neuronal development, learning and memory. Intracellular messengers including Ca^{2+}, IPs, and PKC have been reported to modulate LTP and neuronal development (Lynch and Baudry, 1984), and PCBs inhibit this phenomenon (Altmann *et al.*, 1995; Gilbert *et al.*, 1997). Two congeners representing non-dioxin- (2,2′-DCB; *ortho*-substituted, non-coplanar) and dioxin-like (3,3′,4,4′,5-PeCB; non-*ortho*-substituted, coplanar) classes of PCBs were studied for their effects on Ca^{2+} homeostasis, IP accumulation, and PKC translocation in rat cerebellum or cultured cerebellar granule cells.

PCB effects on Ca^{2+} homeostasis and inositol phosphates. Using Fluo-3 fluorescent probe technique, this laboratory found that both PCB congeners increased cerebellar granule cell $[Ca^{2+}]_i$; 2,2′-DCB was more potent than 3,3′,4,4′,5-PeCB. The increase in $[Ca^{2+}]_i$ produced by these congeners was not transient, but a steady rise was observed with time (Kodavanti *et al.*, 1993a). 2,2′-DCB was a potent inhibitor of mitochondrial (IC50 = 6.17 ± 0.53 μM) and microsomal (IC50 = 7.61 ± 0.35 μM) Ca^{2+} sequestration. 3,3′,4,4′,5-PeCB inhibited Ca^{2+} sequestration by mitochondria (68% of control) and microsomes (72% of control), but the effects were much less than those produced by equivalent concentrations of 2,2′-DCB. Synaptosomal Ca^{2+}-ATPase was inhibited by 2,2′-DCB, but not by 3,3′,4,4′,5-PeCB. We have previously reported that neuroactive chemicals belonging to different categories inhibit Ca^{2+} transporting systems to different extents (Kodavanti *et al.*, 1993b). 2,2′-DCB, but not 3,3′,4,4′,5-PeCB affected basal and carbachol-stimulated IP accumulation in cerebellar granule cells (Kodavanti *et al.*, 1994; Shafer *et al.*, 1996). The effects of 2,2′-DCB on IP accumulation are not due to activation of PKC, but might be due to increased $[Ca^{2+}]_i$ caused by this PCB (Shafer *et al.*, 1996). Further experiments found that 2,2′-DCB, but not 3,3′,4,4′,5-PeCB, was cytotoxic as indicated by a significant increase in LDH leakage at 200 μM after 2 h of exposure. These results indicate that at concentrations where cytotoxicity was not observed, 2,2′-DCB increased $[Ca^{2+}]_i$, altered IP accumulation, and inhibited Ca^{2+} sequestration by intracellular organelles, as well as plasma membrane Ca^{2+}-extrusion process. Although 3,3′,4,4′,5-PeCB appeared to increase $[Ca^{2+}]_i$ to some extent, it was not potent in affecting Ca^{2+} sequestration, Ca^{2+} extrusion, or IP accumulation. Hence, any 3,3′,4,4′,5-PeCB-induced increases in $[Ca^{2+}]_i$ appear to be buffered by intracellular organelles. These results suggested that perturbations in Ca^{2+} homeostasis due to inhibition of membrane Ca^{2+} pumps might play a significant role in the effects of PCBs.

PCB effects on PKC translocation. Subsequent studies have concentrated on the events that could be altered by changes in $[Ca^{2+}]_i$ and IPs. PKC translocation measured as ^{3}H-phorbol ester (^{3}H-PDBu) binding was selected in this respect (Asaoka *et al.,* 1992). PKC is a family of ubiquitous phospholipid-dependent serine/threonine kinases, which play pivotal roles in cellular signal transduction (Nishizuka, 1992). 2,2′-DCB increased PKC translocation in a concentration-dependent manner while 3,3′,4,4′,5-PeCB had no effect. The effect of 2,2′-DCB on PKC translocation was time-dependent and dependent on the presence of external Ca^{2+}, and selected antagonists such as MK-801 (a noncompetitive NMDA antagonist), CPP (competitive NMDA antagonist), CNQX (AMPA antagonist), verapamil (Ca^{2+}-channel antagonist), and tetradotoxin (Na^+ channel antagonist) had no effect on 2,2′-DCB-stimulated PKC translocation (Kodavanti *et al.,* 1994). These results are consistent with previous findings that the non-dioxin-like congener increased Ca^{2+} accumulation and inhibited Ca^{2+} buffering, resulting in increased $[Ca^{2+}]_i$. The increased $[Ca^{2+}]_i$ could cause PKC translocation and lead to other adverse effects such as cytotoxicity. With the dioxin-like congener, there is increased Ca^{2+} accumulation, but little effect was observed on Ca^{2+} buffering resulting in marginal changes in $[Ca^{2+}]_i$ and no change in PKC translocation and the absence of cytotoxicity.

Structure–activity relationships (SAR) of PCB congeners. For SAR studies, PCB congeners were selected on the basis of their presence in the environment (soil and water samples), food, and humans. Effects of three PCB mixtures and 36 PCB congeners were assessed (Table 1). All the PCB mixtures studied increased PKC translocation significantly and in a concentration-dependent manner; Aroclors 1016 and 1254 were more potent than Aroclor 1260. The SAR among PCB congeners revealed: (i) congeners with *ortho*-chlorine substitution such as 2,2′-DCB or *ortho*-lateral (*meta, para*) chlorine substitutions such as 2,2′,5,5′-TeCB and 2,2′,3,5′,6-PeCB were most potent; (ii) congeners with only para-substitution, such as 4,4′-DCB, or high lateral content in the absence of *ortho*-substitution, such as 3,3′,4,4′,5,5′-hexachlorobiphenyl, were not effective; and (iii) increased chlorination was not clearly related to the effectiveness of these congeners (Kodavanti *et al.,* 1995). The relative potency of these congeners is given in Table 1. These results are in agreement with those of Shain *et al.* (1991) and Wong and Pessah (1996).

Our SAR studies suggested that activity of many PCB congeners appeared to be associated with chlorination substitution patterns that favor non-coplanarity, while those with patterns favoring coplanarity were less active. To support this conclusion, studies with a group of chemicals, polychlorinated diphenyl ethers, in which the assumption of a coplanar structure is difficult regardless of degree and pattern of chlorination, were initiated. All of the polychlorinated diphenyl ether congeners studied increased PKC translocation (Kodavanti *et al.,* 1996b), supporting the hypothesis that coplanarity of chlorinated aromatic hydrocarbons seems to reduce the potency *in vitro.* Molecular mechanical studies indicate that the energy required to achieve a coplanar conformation for non-*ortho* PCB is 8 kcal/mol, while it is 16.5 kcal/mol for *ortho*-PCB, suggesting that non-*ortho*-PCBs are more coplanar in nature (Kodavanti *et al.,* 1996). Empirical modeling developed using a nonlinear statistical technique indicated that PCB congeners with intermediate octanol water partition coefficient are also more potent. This model also indicated that position and number of chlorines are important in the activity of PCBs. This model not only predicted the activity of other PCB congeners, but also predicted the activity of commercial as well as environmental mixtures. So far, results from our laboratory have indicated that intracellular messengers are perturbed by PCBs, and these effects are related to the noncoplanarity of the PCB molecule. These features may account for the neurotoxic and certain other toxic effects of PCBs.

GENERAL CONCLUSIONS

The information presented in this symposium indicates that non-coplanar PCB congeners produce biological actions that may contribute to toxicity from environmental exposures to PCBs. The spectrum of activity produced by these congeners has not been fully explored, and the mechanisms by which their known actions are produced are emerging but remain to be fully elucidated. The toxicodynamic interactions between non-coplanar PCBs and the actions produced by coplanar PCBs which bind to the Ah-receptor remain to be investigated. Similarly, the actions and interactions of hydroxylated and other metabolites of PCBs remain to be studied in sufficient depth.

At the present time, it is clear that non-coplanar PCBs alter signal transduction pathways and interrupt intracellular Ca^{2+} homeostasis. A common site of action responsible for all of the actions of non-coplanar PCBs, analogous to the Ah-receptor utilized by coplanar PCBs, has not been found but candidates, including protein kinase C, phospholipases, and the ryanodine receptor, are being evaluated through continuing research.

Regarding structure–activity relationships of PCBs, the effects reported in neuronal preparations, neutrophils, and insulin-secreting cells seem to be related to *ortho*-chlorine substitutions on the PCB molecule. There may be at least three fundamental ways this can come into play, viz., by hindering coplanarity of the rings, by enhancing conformational restriction (more rigid structure, like steroids), and by introducing additional hydrophobic bulk structure in nonlat-

TABLE 1

Relative Potency of PCB Mixtures, PCB Congeners, and Diphenyl Ethers on Protein Kinase C Translocation, Measured as Increases in [^{3}H]PDBu Binding in Rat Cerebellar Granule Cells

Very active (EC50 = <50 μM)	Moderately active (EC50 = 50–100 μM)	Slightly active (EC50 = >100 μM)	Not active (EC50 = NEO)
	PCB mixtures		
	Aroclor 1016	Aroclor 1260	
	Aroclor 1254		
	PCB congeners		
2,2′-DCB	3,3′-DCB	2,4,4′-TCB	4,4′-DCB
2,3,4-TCB	3,5-DCB	2,4,4′,5-TeCB	2,2′,6,6′-TeCB
2,2′,4,6-TeCB	2,2′,6-TCB	2,2′,4,4′,5-PeCB	3,3′,4,4′-TeCB
2,2′,4,6′-TeCB	2,2′,4,4′-TeCB	2,2′,4,4′,6-PeCB	3,3′,4,4′,5-PeCB
2,2′,5,5′-TeCB	3,3′,5,5′-TeCB	2,3′,4,4′,5-PeCB	3,3′,4,5,5′-PeCB
2,2′,5,6′-TeCB	2,3,3′,4,4′-PeCB	2,2′,3,3′,4,4′-HCB	3,3′,4,4′,5,5′-HCB
2,2′,3,3′,4-PeCB	2,2′,3,3′,6,6′-HCB	2,2′,3,3′,5,5′-HCB	2,2′,3,4,4′,5,5′-HeCB
2,2′,3,4,4′-PeCB		2,2′,4,4′,5,5′-HCB	
2,2′,3,5′,6-PeCB		2,3,3′,4,4′,5-HCB	
2,2′,4,5,5′-PeCB		2,2′,3,3′,5,6,6′-HeCB	
2,2′,4,6,6′-PeCB			
2,3,3′,4′,6-PeCB			
	Polychlorinated diphenyl ethers		
2,4,4′-TCDE	4,4-DCDE	3,3′,4,4′-TeCDE	
	Diphenyl ether	2,2′,4,4′,5-PeCDE	
		2,3′,4,4′,5-PeCDE	

Note. EC50 value indicates the effective concentration that increases the control activity by 50%. NEO, no effect observed up to 100 μM. DCB, dichlorobiphenyl; TCB, trichlorobiphenyl; TeCB, tetrachlorobiphenyl; PeCB, pentachlorobiphenyl; HCB, hexachlorobiphenyl; HeCB, heptachlorobiphenyl; DCDE, dichlorodiphenyl ether; TCDE, trichlorodiphenyl ether; TeCDE, tetrachlorodiphenyl ether; PeCDE, pentachlorodiphenyl ether.

eral positions. One or more of these factors may contribute to the various biological activities being investigated. For example, for neuroactive PCBs, the effects on coplanarity may be most important, whereas for estrogenic activity the more steroid-like rigidity may also be an important factor. Additional studies, however, are needed to define the structural correlates of these biological activities.

Non-coplanar congeners represent the major fraction of PCBs found in the tissues of humans and wildlife. It is reasonable to speculate that, even though there is an incomplete understanding of the toxic actions of these congeners, by not considering the potential toxicity of this group of compounds the total health and environmental risks from PCB exposure will not be obtained. Clearly, the use of toxic equivalency factors (TEFs) that are based only on dioxin-like activity is inadequate for estimating the total risk from exposures to PCBs.

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