Altered Biologic Activities of Commercial Polychlorinated Biphenyl Mixtures after Microbial Reductive Dechlorination

Mahmoud A. Mousa, Patricia E. Ganey, John F. Quensen III, Burra V. Madhukar, Karen Chou, John P. Giesy, Lawrence J. Fischer, and Stephen A. Boyd

1Department of Crop and Soil Sciences; 2Department of Pharmacology and Toxicology; 3Department of Pediatrics and Human Development; 4Department of Animal Sciences; 5Department of Zoology; 6Institute of Environmental Toxicology, Michigan State University, East Lansing, Michigan

The reductive dechlorination of polychlorinated biphenyls (PCBs) by anaerobic bacteria has recently been established as an important environmental fate of these compounds. This process removes chlorine directly from the biphenyl ring with replacement by hydrogen, resulting in a product mixture in which the average number of chlorines per biphenyl is reduced. In this study, dechlorination of commercial PCB mixtures (Aroclors 1242 and 1254) by microorganisms eluted from PCB-contaminated sediments of the River Raisin (Michigan) and Silver Lake (Massachusetts) caused a depletion in the proportion of highly chlorinated PCB congeners and an accumulation of lesser-chlorinated congeners. Dechlorination occurred primarily at the meta and, to a much lesser extent, para positions of biphenyl. The concentrations of the coplanar congeners including 3,3',4,4',5-pentachlorobiphenyl, the most potent dioxinlike congener, were significantly lowered by reductive dechlorination. Microbial reductive dechlorination of commercial PCB mixtures caused a substantial reduction in biologic activity in several instances. It significantly lowered or eliminated the inhibitory effects of Aroclors on fertilization of mouse gametes in vitro. Similarly, the dechlorinated product mixtures had substantially lower ethoxyresorufin-O-deethylase induction potencies and showed less ability to induce activating protein 1 transcription factor activity as compared to the unaltered Aroclors. In other assays the same dechlorinated product mixtures demonstrated biologic activities similar to the nondechlorinated Aroclors, including the ability of PCB mixtures to stimulate insulin secretion and cause neutrophil activation.

The data presented here establish that the biologic activities of commercial PCB mixtures are altered by microbial reductive dechlorination and that an assessment of their toxic potential requires an array of tests that include the different mechanisms associated with PCBs. — Environ Health Perspect 106(Suppl 6):1409–1418 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl6/1409-1418mousa/abstract.html

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Polychlorinated biphenyls (PCBs) are ubiquitous industrial organic chemical contaminants distributed throughout the global ecosystem (1–7). Commercial PCBs (e.g., Aroclors) are complex mixtures containing about 80 to 90 of a possible 209 congeners that differ in the number and positioning of chlorines on the biphenyl ring structure. Approximately 1.4 billion pounds of PCBs have been produced worldwide, and several hundred million pounds have been released to the environment (8). PCBs produce a broad spectrum of toxicities and biologic activities in laboratory animals and humans and produce a variety of ecosystem-level effects. In this paper we evaluate changes in the biologic activities of commercial PCB mixtures that occur as a result of microbial reductive dechlorination, which is now recognized as an important in situ process (9,10). Reductive dechlorination removes chlorine from biphenyl with replacement by hydrogen, resulting in a product mixture in which the average number of chlorines per biphenyl is substantially diminished. Collectively, our recent studies demonstrate that an accurate assessment of the risk posed by PCBs in the environment requires evaluation of the biologic activities of the mixtures that occur in situ, which may be substantially different from the parent mixtures because of anaerobic microbial processes (11). Furthermore, the use of multiple biologic end points is critical for realistic evaluation of the toxicity of microbiologically dechlorinated PCB mixtures.

Exposure to complex PCB mixtures has resulted in numerous adverse health effects in exposed animals (12). Examples of some effects linked to PCB exposure include wasting syndromes, body- and organ-weight abnormalities (13–15), reproductive failure (16–22), eggshell thinning (23,24), immune suppression and greater susceptibility to infectious diseases (25), carcinogenicity (26), increased mortality (27), and suppression of population numbers (22). Pre- and postnatal exposure of laboratory animals to PCBs resulted in changed birth weight of offspring (28,29), retarded learning and loss of short-term memory (30–32), poor discrimination-reversal learning (33,34), and variability in reproductive (28,29), hematologic, and serum biochemical parameters (35). Exposure of male rodents to PCBs also caused improper implantation of fertilized eggs in non-PCB-exposed females to which they were mated (36–38). Gestational and neonatal exposure of rodents to PCBs...
resulted in embryonic abnormality and reduced fertility (28,29,39,40).

Different structural classes of PCB congeners elicit different toxicologic responses. Coplanar PCB congeners (non- or mono-ortho-substituted PCBs) exhibit dioxinlike activity. They induce cytochromes P450 and cause increased liver weight, thymus atrophy, and diminished body weight (12,41,42). Exposure of rats to coplanar PCB congeners causes a marked decrease in vitamin A concentration in blood and liver and a subsequent reduction in the levels of retinol and thyroxin (43). Inhibition of leukotriene production by opsonized zymosan in human polymorphonuclear granulocytes (PMN) was affected by coplanar congeners such as 3,3',4,4'-tetrachlorobiphenyl (44). The coplanar PCB congeners express their dioxinlike effects through aromatic hydrocarbon receptor (Ah-R)-mediated processes (45).

Noncoplanar PCBs may exert different types of toxicity. Evidence is mounting that neurotoxicity results from noncoplanar congeners that influence catecholamine levels in nerve tissues (46–48). Signal transduction systems can be altered by noncoplanar congeners, and this may adversely impact a variety of critical biologic functions including the nervous system (49,50) and the immune system (25,51–53).

Microbial reductive dechlorination of commercial PCB mixtures was first inferred from altered congener profiles in anaerobic sediments from the Upper Hudson River, New York, in which a depletion in more heavily chlorinated congeners and an enhancement of lower chlorinated congeners was observed (54,55). This was subsequently confirmed in laboratory studies in which Aroclors were dechlorinated when added to non-PCB-contaminated Hudson River sediments that were then inoculated with anaerobic microorganisms eluted from PCB-contaminated Hudson River sediments (9,56). In situ PCB dechlorination has been documented at numerous sites including the Hudson River, Silver Lake (SL; Massachusetts), the Sheboygan River (Wisconsin), the Hoosic River (Massachusetts), and the River Raisin (RR; Michigan) (10).

Although in situ PCB dechlorination is common, the extent of dechlorination varies considerably among sites, ranging from <10 to >90% removal of chlorine in the meta and para positions of biphenyl (10). Dechlorination at the ortho position does not generally occur. Based on chromatographic profiles of dechlorinated product mixtures, several fundamental dechlorination processes have been described (10) that may occur singly or in combination in the environment. These processes are believed to result from differences in the congener specificities of distinct species or strains of dechlorinating microorganisms. Processes M and Q are, respectively, the most extensive individual meta and para dechlorination processes because they do not require that a chlorine be adjacent to the position dechlorinated. All other dechlorination processes have this requirement and hence remove less chlorine. Process M removes chlorine from the meta positions (3',5') of biphenyl and appears most widely distributed in anaerobic sediments. Process Q removes para (4,4') chlorines and is less frequently observed. Process C occurs when both processes M and Q are active, producing the most extensively dechlorinated product mixture. It results in the accumulation of primarily ortho-substituted mono- and dechlorinated congeners. Differences in the activity of each of these dechlorination processes can produce distinct mixtures of PCB congeners as end products whose compositions are vastly different from the technical Aroclor mixtures released into the environment. Such changes in the congener profiles are likely to shift the spectrum of biologic activity and toxicity of the PCB mixture. Knowledge of the biologic activities of dechlorinated PCB product mixtures that commonly occur in nature is necessary for an accurate assessment of the risks they pose. We report here an evaluation of the changes in biologic activities of commercial PCB mixtures that occur as a result of microbial reductive dechlorination.

Materials and Methods

Production of Dechlorinated Product Mixtures

Large-scale anaerobic microbial incubations were performed to produce sufficient quantities of dechlorinated PCB mixtures from Aroclors 1242 and 1254 for use in the different biologic assays. Dechlorinating microorganisms were eluted from PCB-contaminated RR and SL sediments where in situ dechlorination had occurred (10). In an anaerobic glove chamber, approximately 1 liter sediment was placed in a 2-liter Erlenmeyer flask previously flushed with O2-free N2. Revised anaerobic mineral medium (RAMM) (57) was added to the flask, and the contents were mixed vigorously for about 2 min then left to settle. The supernatants containing the RR or SL organisms were decanted into separate flasks (previously flushed with O2-free N2) and subsequently used as the inocula. Clean sediment (PCB-free) was obtained from the Red Cedar River (Okemos, Michigan), air dried, and passed through a 2-mm sieve. In an anaerobic glove chamber, 75 g sediment was dispensed into 100-ml serum vials, slurred with 60 ml RAMM, and sealed with butyl rubber stoppers. The slurry was incubated at room temperature in the anaerobic glove chamber for about 2 weeks until methane was detected in the headspace. The slurries were then autoclaved (2 hr at 130°C) and returned to the anaerobic chamber, amended with PCBs (Aroclors 1242 and 1254, 600 mg/g sediment) and inoculated with the dechlorinating microorganisms (20 ml, RR or SL). The vials were crimp-sealed, vortexed, and then incubated in an anaerobic glove chamber at room temperature.

In addition to the actively dechlorinating microorganisms, a control containing auto-claved (deactivated) inoculum together with PCBs and a control containing inoculum with no PCBs were prepared and incubated as above. Samples were withdrawn periodically for PCB analysis to monitor the dechlorination process. After the dechlorination activity reached a plateau (approximately 9 months), the incubation was terminated and the contents of each 100-ml vial were transferred into 1-liter bottles. Extraction and congener-specific quantification of PCBs were as described previously (11). Briefly, PCBs were extracted twice with 500 ml acetone and three times with 500 ml hexane:acetone (9:1). The extraction solvents were evaporated under N2 and the residues were dissolved in 100 ml hexane. The hexane solution was first washed with concentrated sulfuric acid (3 × 25 ml), then with 2% sodium chloride solution (2 × 50 ml), then passed over a 50-ml Florisil copper column contained in a 100-ml burette. The total PCB concentration and the identity and concentration of the individual congeners were determined by gas chromatography (9). The congener composition was expressed as molar percentage of the total moles of biphenyl in each sample. Non-dechlorinated Aroclors 1242 and 1254 and their molar equivalent of dechlorinated PCBs were made into solutions in the proper solvent for each biologic assay.
A separate analysis for coplanar PCB congeners and for polychlorodibenzo-dioxin and -dibenzo-furan (PCDD/PCDF) contamination was performed as described by Quensen and colleagues (58). The coplanar congeners determined (with International Union of Pure and Applied Chemistry numbers) were 2,3,4,5,6-chlorodibenzofuran (CB) (117); 2,3,3′,4,4′-CB (110); 3,4,4′,5-CB (81); 3,3′,4,4′-CB (77); 2,3,3′,4,5-CB (123); 2,3,3′,5,5′-CB (118); 2,3,3′,4,5-CB (114); 2,3,3′,4′-CB (105); 3,3′,4,4′-CB (126); 3,3′,2,3,4,4′-CB (157); 2,3,3′,4,4′-CB (156); 2,3′,3′,5,5′-CB (167); 3,3′,4,4′,5,5′-CB (169); and 2,3,3′,4,4′,5,5′-CB (189). The mass selective detector was operated in electron impact selected ion monitoring mode. A CompacP CP SIL 5/C18 (Hewlett-Packard, Wilmington, Delaware) (100 m × 0.25 mm id 0.10-mm film) was used to achieve near-complete resolution of the target congeners. The instrument detection limit (IDL) for these analyses ranged from 1.5 to 8.0 pg in the injected sample. The reported quantification limit was three times the IDL or 4.5 to 24 pg in the injected sample.

**Biologic Testing of Dechlorinated and Nondechlorinated Polychlorinated Biphenyls**

Biologic activities of the PCB mixtures before and after dechlorination were evaluated using an array of biologic end points consisting of *in vitro* fertilization, ethoxyresorufin-O-deethylase (EROD) induction, induction of activated protein (AP)-1 transcription activity, insulin release, and neutrophil activation.

**In Vitro Fertilization.** Sperm and eggs were collected from mice and exposed *in vitro* to nondechlorinated and dechlorinated PCBs and to controls that contained no PCBs. Sperm were collected from epididymides of B6D2F1 male mice (Jackson Laboratory, Bar Harbor, Maine). This crossbreed from C57BL/6J females and DBA/2J males has been responsive to dioxin and PCBs in CYP1A induction (59). The *in vitro* fertilization assay has been described previously (12) and modified as follows. Epididymal sperm samples were collected in 1 ml BMOC-3 medium containing the test material (PCBs, dechlorinated PCBs, or no PCBs) and incubated for 1 hr. Eggs from B6D2F1 females were also collected in 1 ml BMOC-3 medium containing the test material and incubated for 1 hr. The eggs were then inseminated with 50 μl sperm suspension at final sperm concentrations of 0.5 to 1.5 × 10⁴/ml. Twenty-five to 27 hr after insemination, eggs at the one- or two-cell stage with two pronuclei and a second polar body were considered fertilized. Fragmented, degenerated, and one-cell eggs without multiple nuclei were considered nonfertilized. The results of the *in vitro* fertilization were analyzed by the Chi-square procedure (MINITAB Inc., State College, Pennsylvania) at the 0.05 significance level. Concentrations of 10 and 20 ppm of nondechlorinated Aroclor 1242 and 1254 and their molar equivalent of the dechlorinated PCBs were tested in this assay. The designated concentration of the dechlorinated PCBs (in units of parts per million) refers to the total molar concentration of the starting material, not the actual weight of the dechlorinated products. Despite being added in excess of their water solubilities, PCBs remained in solution because of their association with serum components (60).

**Ethoxyresorufin-O-deethylase Activity.** The dioxinlike activity of dechlorinated and nondechlorinated Aroclors 1242 and 1254 was evaluated using the EROD induction assays in H4IIE rat hepatoma cells. The potencies of dechlorinated and nondechlorinated PCBs as inducers of EROD activity in H4IIE cells were determined from the median effective dose values of full dose–response curves and expressed as 2,3,7,8-tetrachlorodibenzop-p-dioxin (2,3,7,8-TCDD) equivalents (TEQs) as described previously (61). Briefly, H4IIE cells were added to 96-well plates at a volume of 250 μl and cell density of 2 × 10⁴ cells/ml and dosed with Aroclors, dechlorination products, control solutions, or 2,3,7,8-TCDD standards in 5 μl isooctane. Aroclors were added at 0.04 to 2.5 μg/ml, Dechlorination products were added at the total molar equivalent of 0.04 to 2.5 μg original Aroclor/well and 2,3,7,8-TCDD was added at 0.3 to 20 pg/ml. Control wells received 5 μl isooctane. Cells were incubated for 72 hr, and the EROD activity was analyzed fluorometrically (61).

**Activating Protein-I Activity.** Another end point used to evaluate the effects of reductive dechlorination of PCBs was induction of AP-1 transcriptional factor, which has been implicated in the neoplastic promotion process (62–66). A WB–F344 rat liver epithelial cell line (WB) was used to determine effects on AP-1-regulated gene expression. The WB cell line was derived from liver of male Fischer 344 rats (67). The cells were routinely cultured in modified minimal essential medium (MEM) with 10% fetal calf serum (FCS) in a humidified atmosphere (95% O₂/5% CO₂) at 37°C. The luciferase plasmid (provided by M.J. Karin, University of California at San Diego, San Diego, California) contained two AP-1 binding sites in a head-to-tail orientation followed by the luciferase coding sequence. For stable expression of the reporter gene, the cells were cotransfected with pSV2 neoplasmin DNA carrying the neomycin resistance gene (American Type Culture Collection, Rockville, Maryland). Following transfection, the WB cells were selected for neomycin resistance in the presence of gentamicin (500 μg/ml). One clone that expressed high luciferase activity was selected for the study.

Prior to treatment, cells were trypsinized and seeded into 12- or 24-well tissue culture dishes in the MEM + 10% FCS with G-418 (500 μg/ml). Twenty-four hours later the medium was changed and replaced with fresh MEM containing 0.2% FCS serum and 0.1% fatty acid-free bovine albumin. This shift to low serum was necessary to decrease the serum-induced effects on AP-1. After 24 hr the medium was changed again and replaced with the same low serum medium containing the test materials (2 μg/ml) dissolved in dimethyl sulfoxide (DMSO). After treatment for 12 hr the medium containing PCBs was removed, and the cultures were rinsed twice with phosphate-buffered saline. Cells were then lysed with 100 μl lysis buffer (Promega Corporation, Madison, WI). Luciferase activity of the lysates was measured in a Berthold Luminometer (EG&G Berthold, Wildbad, Germany) and normalized to total cell protein. Protein concentrations of the samples were assayed using Bio-Rad DC (Hercules, California) protein reagents. Each experiment was performed in triplicate, and the mean value was reported as a percent of the control.

**Insulin Release.** Insulin release from the insulin-producing cell line RINm5F produced by dechlorinated and nondechlorinated PCBs was measured as previously reported (68). Nonconfluent cells were incubated in fresh RPMI 1640 media for 30 min prior to addition of PCB mixtures (dissolved in DMSO) to produce 10 ppm of unaltered PCBs and the molar equivalent of the dechlorinated mixtures. The treated cells were incubated for 30 min and a sample of media was removed for insulin analysis by radioimmunoassay. Cells were
washed and collected for DNA analysis. Insulin released into media was expressed as nanograms insulin per microgram DNA.

**Neutrophil Activation.** Induction of neutrophils to produce superoxide anion radical ($O_2^{\cdot-}$) was another assay used to assess changes in the biologic activity of PCBs resulting from microbial dechlorination. Animals were used in accordance with guidelines set forth by the All-University Committee on Animal Use and Care at Michigan State University (East Lansing, Michigan). Neutrophils were isolated from the peritoneum of rats by glycogen elicitation as described previously (69), and suspended ($2 \times 10^9$)/ml in Hanks' balanced salt solution. Cells were then exposed to nondechlorinated Aroclors at a concentration of 10 $\mu$g/ml or to dechlorinated PCBs at an equivalent molar concentration. Neutrophils were incubated with the PCB mixtures or dechlorination products at 37°C for 30 min, and after the first 5 min phorbol myristate acetate ([PMA] 20 ng/ml) was added to the incubation mixture. Production of $O_2^{\cdot-}$ over the 30-min period was determined from superoxide dismutase-sensitive reduction of cytochrome $c$ (70).

**Results and Discussion**

Long-term incubations under anaerobic conditions were used to produce sufficient quantities of dechlorinated PCB mixtures for evaluation of their biologic activities in comparison to the unaltered parent Aroclors. We used two commercial PCB mixtures, Aroclor 1242 and Aroclor 1254, and microorganisms from two PCB-contaminated sites (RR and SL) where *in situ* dechlorination has occurred. The microbially dechlorinated PCBs produced in laboratory incubations are representative of dechlorinated product mixtures that actually occur in nature (10,71). The biologic activities and toxicities of the original and dechlorinated PCB mixtures were assessed using an array of end points measured *in vitro* including fertilization,

![Histograms representing the congener profiles of commercial PCB mixtures (Aroclors) before and after microbial reductive dechlorination.](image-url)

Figure 1. Histograms representing the congener profiles of commercial PCB mixtures (Aroclors) before and after microbial reductive dechlorination. Each bar in the histogram corresponds to a gas chromatographic peak. The peak numbers correspond to the order in which the congeners were eluted; lower numbered peaks generally represent less chlorinated congeners. Congener content(s) in each peak are listed by Mousa et al. (60). (A) Aroclor 1242 and (B) Aroclor 1254 were dechlorinated by microorganisms eluted from PCB-contaminated sediments of RR or SL.
EROD induction, induction of AP-1 transcription activity, insulin release, and neutrophil activation.

**Microbial Dechlorination of Polychlorinated Biphenyls**

Each inoculum/Aroclor combination produced a different mixture of dechlorinated PCBs. The product mixtures were enriched in lesser chlorinated congeners and depleted of higher chlorinated congeners relative to the parent Aroclor (Figure 1). Dechlorination occurred primarily at the *meta* positions of biphenyl and to a lesser extent at the *para* positions; no *ortho* dechlorination was observed. Predominant PCB congeners dechlorinated and those produced for each Aroclor/inoculum combination are given in Table 1. The predominant congeners in the dechlorinated product mixture emanating from Aroclor 1242 were mono- and dichlorobiphenyls whereas those from Aroclor 1254 were di- and chlorobiphenyls. Composition of the dechlorinated product mixtures are summarized below. A more complete characterization of the Aroclors and dechlorinated product mixtures has been given by Quensen and colleagues (58).

The extent of dechlorination of complex PCB mixtures is conveniently expressed in terms of the average number of *meta* and *para* chlorines per biphenyl in the mixture as a whole, as *ortho* dechlorination is not commonly observed. Dechlorination of Aroclor 1242 by the RR organisms removed 56% of the *meta* and *para* chlorines with no evidence of *ortho* chlorine removal. Dechlorination by the RR microorganisms occurred primarily from the *meta* positions. The predominant dechlorination products (Table 1), which contained chlorine primarily in the *ortho* and *para* positions, accounted for 15.5 mol% of the parent Aroclor but were 68.8 mol% of the dechlorination products. Less prominent products (those increasing by <5 mol% of the total molar PCB concentration) comprised 11.1 mol% of the parent Aroclor and 24.5 mol% of the dechlorination products [Mousa et al. (11) discuss specific congeners]. Such a dechlorination pattern has been described as process M and is characterized by the removal of flanked *meta* chlorines (those which have adjacent positions occupied by chlorine) as well as nonflanked *meta* chlorines (10).

Dechlorination of Aroclor 1242 by SR microorganisms was less extensive than by RR microorganisms, with an average of 40% of the *meta* and *para* chlorines being removed. The predominant congeners produced (Table 1) totaled only 11.4 mol% of the parent Aroclor and 36.3 mol% of the dechlorination products. Less prominent products comprised 29.8 mol% of the parent Aroclor and 54.2 mol% of the dechlorination products. Dechlorification by SR microorganisms was less extensive than by RR microorganisms but formed more 2,3',5-triCB, 2,3',4-triCB, and 2,4',5-triCB than did RR microorganisms. This indicated that SR microorganisms were less able to remove nonflanked *meta* chlorines than were RR microorganisms.

Dechlorination of Aroclor 1254 by RR microorganisms removed an average of 54% of the *meta* and *para* chlorines with no evidence that any *ortho* chlorines were removed. The predominant products (Table 1) totaled only 1.0 mol% of the parent Aroclor but were 69.3 mol% of the dechlorination products. PCB congeners comprising these products contained chlorine mainly at the *ortho* or *ortho* and *para* positions, which indicated that *meta* chlorines were removed from both flanked and nonflanked *meta* positions. Less prominent products comprised 2.4 mol% of the parent Aroclor and 17.4 mol% of the dechlorination products.

Dechlorination of Aroclor 1254 by SR microorganisms was less extensive than that by RR microorganisms, with an average of 44% of the *meta* and *para* chlorines removed. The predominant products (Table 1) accounted for only 2.1 mol% of the parent Aroclor but 52.3 mol% of the dechlorination products. Less prominent products represented 1.9 mol% of the parent Aroclor and 24.5 mol% of the dechlorination products. Congeners containing nonflanked *meta* chlorines made up a greater proportion of the SL dechlorination products as compared to the RR dechlorination products. SL microorganisms produced more 2,3',6-triCB/2,3,6-triCB, 2,3',4-triCB, and 2,2',4,5'-tetrachlorobiphenyl than RR microorganisms. These differences again indicated that SL microorganisms were less capable of removing nonflanked *meta* chlorines than were the RR microorganisms.

The concentrations of coplanar congeners in the PCB mixtures were significantly reduced after reductive dechlorination (Figure 2). Dechlorination

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**Table 1.** Predominant PCB congeners produced or reduced via reductive dechlorination of Aroclors 1242 and 1254 by microorganisms from River Raisin and Silver Lake. The congeners listed increased by >5% of the total molar concentration of the PCB mixture.

| Congeners produced | Aroclor 1242 |  | Aroclor 1254 |  |
|---------------------|--------------|------------------|--------------|------------------|
| RR                  | SL           | RR              | SL           |                   |
| 2-CB*               | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a |
| 2,4'-diCB/2,3-diCB*a | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a |
| 2,2',4-triCB#       | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a |
| 2,3',4-triCB*       | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a | 2.2'-diCB/2,6-diCB*a |

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*Congeners listed together coeluted and were not quantified independently.

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**Figure 2.** The concentration of coplanar PCB congeners significantly decreased after reductive dechlorination of Aroclors 1242 and 1254. The concentrations of the following coplanar congeners in the nondechlorinated (autoclaved) RR- and SL-dechlorinated Aroclors 1242 and 1254 are depicted: 81 (3,4,4',5-tetrachlorobiphenyl), 77 (3',4',4'-tetrachlorobiphenyl), 114 (2,3,4',5-pentachlorobiphenyl) and 126 (3',4',4',5-pentachlorobiphenyl). Adapted from Quensen et al. (58).
of Aroclors 1242 and 1254 by RR and SL microorganisms lowered the concentrations of both the non- and mono-ortho-substituted congeners, often by at least 10-fold and in some cases > 100-fold, as in the case of dechlorination of Aroclor 1242 by RR microorganisms (Figure 2). For example, dechlorination reduced the concentration of 3,3′,4,4′,5-pentaCB (congener 126), the most potent dioxin-like congener (61, 72), by one to two orders of magnitude for Aroclor 1242 (SL and RR, respectively) and by nearly one order of magnitude for Aroclor 1254 (58).

Biologic Testing of Dechlorinated and Nondechlorinated PCBs

In Vitro Fertilization of Mouse Gametes. Exposure of gametes to the nondechlorinated Aroclors resulted in a high percentage of degenerated eggs and a low percentage of fertilized eggs. In the absence of PCBs, few eggs (1–7%) degenerated and most eggs (83–86%) were fertilized (Figure 3).

Dechlorination of Aroclor 1242 markedly reduced or eliminated the adverse effects of PCBs on fertilization using mouse gametes in vitro (Figure 3). At the highest concentration (20 ppm), unaltered Aroclor 1242 caused all eggs to degenerate and completely inhibited fertilization. However, in the presence of equivalent molar concentrations of the dechlorinated products, egg degeneration was not different from that in the controls containing no PCBs. Furthermore, the percent of fertilized eggs increased from zero to 63% and zero to 21% in eggs exposed to RR and SL dechlorinated products, respectively. At 10 ppm of the parent Aroclor 1242, few eggs degenerated but only 35% were fertilized. Dechlorination by RR microorganisms resulted in a fertilization rate that was similar to that of the control containing no PCBs, indicating complete alleviation of the toxic effects on fertilization at this concentration. In the presence of 10 ppm of SL dechlorination products, the proportion of fertilized eggs did not differ significantly from that of nondechlorinated Aroclor 1242.

Dechlorination of Aroclor 1254 resulted in a similar reduction in the toxic effects of PCBs on mouse gametes (Figure 3). Unaltered Aroclor 1254 at the higher concentration (20 ppm) caused all eggs to degenerate and hence no fertilization occurred. However, when exposed to the RR and SL dechlorination products at the equivalent dose, only 21 and 16% of the eggs degenerated, and 8 and 31% of the eggs were fertilized, respectively. Exposure to 10 ppm of the parent Aroclor 1254 also resulted in considerable egg degeneration (66%) and low egg fertilization (21%).

Dechlorination of Aroclor 1254 by RR microorganisms, as with Aroclor 1242, eliminated all observed toxic effects at the equivalent dosage. Dechlorination of Aroclor 1254 by SL microorganisms achieved a similar effect, resulting in fewer degenerated eggs and an increase in the percentage of fertilized eggs.

Ethoxyresorufin-O-Deethylase Activity. The Ah-R-mediated activity of the nondechlorinated and dechlorinated Aroclors was evaluated using H4IIE rat hepatoma cell bioassay for EROD induction potency (58). The potencies of the PCB mixtures were standardized to that of 2,3,7,8-TCDD to yield TCDD equivalents (TEQ assay). The measured values were compared to calculated TCDD equivalents (TEQcal), which were based on the measured coplanar congener concentrations and their individual TCDD equivalency factors, as summarized in Table 2. As expected from the decreased amounts of coplanar PCBs, the measured TCDD equivalents (TEQs) determined using the H4IIE bioassay demonstrated substantial reduction in the dioxinlike potencies of PCB mixtures as a result of microbial dechlorination. The decreases in EROD induction potencies depended on the extent of removal of non- or mono-ortho-substituted congeners. This is revealed by the correspondence between the TEQs determined from the H4IIE bioassay and those calculated.

Table 2. TEQ assay and TEQcalc for unaltered and microbially dechlorinated Aroclors 1242 and 1254. PCB dechlorinating organisms were obtained from the River Raisin and Silver Lake.

| Sample          | TEQ assay | TEQcalc |
|-----------------|-----------|---------|
| Aroclor 1254    |           |         |
| Nondechlorinated| 7.5       | 7.8     |
| SL dechlorinated| 2.1       | 1.6     |
| RR dechlorinated| <0.06c    | 1.0     |
| Aroclor 1242    |           |         |
| Nondechlorinated| 3.1       | 5.7     |
| SL dechlorinated| <0.06c    | 0.078   |
| RR dechlorinated| <0.06c    | <0.06f  |

*TEQ assay values were determined using H4IIE rat hepatoma cell bioassays for EROD induction potencies.

*TEQcalc values were calculated from molecular concentrations of coplanar congeners and their TCDD equivalency factors. cLess than minimum detection limit. Data from Quensen et al. (58).
from the coplanar congener concentrations. Agreement between TEQcalc and TEQ assay also indicated no unexpected synergistic interactions among components of the dechlorination mixtures. Generally dechlorination by RR microorganisms reduced the EROD induction potencies of the Aroclor product mixture to somewhat greater extents than that caused by SL microorganisms. It is important to note that the extracts were screened for PCDD/PCDF contamination. No 2,3,7,8-substituted tri- or pentachlorodibenzodioxins or -dibenzofurans were detected at concentrations above the reported qualification limits of 20 to 50 pg/ml. Calculated TEQs indicated that these components would contribute <1% of the TEQs measured in the bioassay. These results from in vitro examination of EROD induction are consistent with results observed in vivo. Treatment of mice with extracts from PCB-contaminated soils from which coplanar PCBs had been OD by treremo mechanica caused less induction of EROD Activity with extracts from PCB-contaminated soil that had not been stripped of the dioxinlike congeners (73).

Activating Protein-1 Activity. The transcription factor AP-1 is a protein that regulates gene expression and has been implicated in tumorigenesis. A reporter gene (luciferase containing AP-1 DNA recognition sequences [response elements] transfected into WB cells) was used to evaluate the activity of dechlorinated and nondechlorinated PCBs. The unlabeled Aroclor 1254 caused a 2- to 3-fold increase in induction of AP-1 transcription factor as measured by the luciferase expression of WB cells. Dechlorination of Aroclors by either RR or SL microorganisms caused significant reduction in the induction of the AP-1 activity similar to controls without PCBs (Figure 4). The observation that PCBs induce the transcription factor AP-1 provides insights into possible cellular interactions of these agents in the modulation of gene expression that leads to neoplastic transformation. Results with individual tests with Aroclor 1242 were insensitive because the potency for induction of AP-1 was insufficient to observe a decline caused by dechlorination (data not shown).

Insulin Release. Polychlorinated biphenyls stimulate release of insulin from the clonal cell line RINm5F (68). The mixture produced from dechlorination of Aroclor 1242 by microorganisms from SL released approximately 70% more insulin than the nondechlorinated Aroclor mixture whereas the product of dechlorination by RR microorganisms did not affect insulin release (Figure 5). Insulin release was unaffected by dechlorination of Aroclor 1254 by either SL or RR microorganisms. Previous results indicated that noncoplanar congeners having ortho-substituted chlorine on one or both of the biphenyl rings are more active than coplanar congeners in releasing insulin from RINm5F cells (68). Thus, results observed with the dechlorination product mixtures, which comprise predominantly ortho-substituted congeners, are consistent with earlier observations.

Neutrophil Activation. Exposure of neutrophils to Aroclor 1242 in the presence of PMA increased O2− production (69). O2− generation by neutrophils in response to Aroclors 1242 or 1254 was not affected by dechlorination (Figure 6). Because dechlorination results in a mixture with a greater percentage of ortho-substituted congeners, these data are consistent with results of previous studies demonstrating that ortho-substituted congeners, and not meta-substituted congeners, activate rat neutrophils to generate O2− (51,69,74).

Conclusion

The process of reductive dechlorination of PCBs causes an important change in the congener profile and a concomitant change in the spectrum of biologic activities. Because this process produces a mixture of lightly chlorinated congeners having one or more ortho chlorines, biologic activities associated with lower chlorinated congeners are retained, whereas activities associated with Ah-R-binding congeners are diminished. The results described above document the need for extensive evaluation of the biologic activities of microbially transformed PCBs. The five assays used in these studies represent a variety of mechanisms by which PCBs act, and such are a useful battery, but they do not encompass all of the possibilities. For example, no measure of estrogenlike activity of the dechlorination products was assessed. This is an issue of current interest for PCBs and PCB-like chemicals, and evidence suggests that some ortho-substituted PCB congeners, as well as hydroxylated metabolites, have activity at estrogen receptors (75,76). Furthermore, these five assays examined only in vitro effects of the dechlorinated mixtures, and clearly there is a need to assess the effects that occur upon in vivo exposure to microbial products of PCB dechlorination. In addition, dechlorinated congeners are more...
susceptible to oxidative metabolism, which leads to the formation of additional compounds and mixtures whose biologic activities will require characterization. Given the multitude of effects of PCBs, the number of congeners and their metabolites found in the environment, and the diverse mechanisms by which PCB congeners can produce changes in biologic function, it is not enough to evaluate effects of environmental products. It is necessary to understand the mechanisms by which PCBs exert their biologic actions more completely. It is likely that the dichotomous categorization of mechanisms (and consequently congeners) as Ah-R-mediated or non-Ah-R-mediated is too simplistic and not reflective of nature. A better understanding of the mechanisms of action of individual PCB congeners, including those found in the environment as a consequence of microbial degradation, will provide a basis for more accurate risk assessment.

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