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Stimulatory effects of a microbially dechlorinated polychlorinated biphenyl (PCB) mixture on rat uterine contraction *in vitro*

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Abstract

Microbially mediated reductive dechlorination has been advocated as the first part of a two-stage (anaerobic/aerobic) biotreatment process for polychlorinated biphenyls (PCBs) in sediments, and is generally viewed as a detoxication process. However, previous studies suggest that microbial dechlorination increases ability to stimulate uterine contractions compared with the original PCB mixtures. Here, we investigate the composition and uterotonic activity of the commercial PCB mixture Aroclor 1260 before and after incubation with microorganisms eluted from PCB-contaminated sediment of the Hudson River. Incubation with microorganisms resulted in a partially dechlorinated mixture (HR1260) dominated by *ortho*-substituted PCBs with four or fewer chlorines per biphenyl. Aroclor 1260 that had not been incubated with microorganisms had no significant effect on contraction frequency of rat uterine strips (gestation day 10) *in vitro*, whereas HR1260 dramatically increased contraction frequency to $718 \pm 134\%$ of the basal rate at a total PCB concentration of 70 μM ($p < 0.05$). The microbial dechlorination increased 2,2',4,4'-tetrachlorobiphenyl and one or more of four congeners that co-eluted during chromatography (2,3,3',5-tetrachlorobiphenyl, 2,3',4,5-tetrachlorobiphenyl, 2,2',4,4',6-pentachlorobiphenyl and 2,2',4,5',6-pentachlorobiphenyl) to 24 and 8 mol %, respectively. However, the uterotonic activities of the latter congeners were modest when evaluated either solely or in a reconstituted mixture and could not fully account for the uterotonic activity of HR1260. Nonetheless, the relative abundance of congeners with three or fewer chlorines increased to 14 mol % as a group in HR1260, suggesting that these congeners collectively contribute to the uterotonic activity even though the abundance of any one congener in this group was less than 5 mol %.

Keywords

polychlorinated biphenyl (PCB); Aroclor 1260; uterine contraction; reproduction; bioremediation; microbial dechlorination

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1. Introduction

Polychlorinated biphenyls (PCBs) are widespread contaminants that bioaccumulate through the food web because of their lipophilicity and persistence (Francis, 1994; Kimbrough, 1995; Wassermann et al., 1979). PCBs were produced and used commercially as mixtures of chlorinated biphenyl (CB) congeners with varying degrees of chlorine substitution of the biphenyl rings. Aroclors are one series of commercial PCBs, consisting of several distinct products with different congener compositions and total chlorine contents (indicated by the last two digits of their four digit designations). The toxicological activities of PCB mixtures and individual PCB congeners are important for understanding the mechanisms by which PCBs induce adverse effects and for developing effective health risk assessments of PCBs (Hansen, 1998; Safe, 1994).

Exposure to certain commercial PCB mixtures has been linked to premature birth and miscarriage in women, suggesting that labor could initiate due to stimulatory action of PCBs (Taylor et al., 1984). Our previous studies revealed that the commercial PCB mixtures Aroclor 1242 and Aroclor 1248, and the *ortho*-substituted PCB congeners 2,4,6-CB and 2,2',4,4'-CB, directly stimulated muscle contraction frequency of pregnant rat uterus *in vitro* (Bae et al., 2001; Tsai et al., 1996). However, neither of the co-planar PCBs 3,3',4,4'-CB or 3,3',4,4',5-CB, nor the highly chlorinated congener 2,2',5,5',6,6'-hexachlorobiphenyl, stimulated contraction frequency with acute exposure (Tsai et al., 1996). Therefore, the number and position of chlorines were important factors in the stimulatory effects of PCBs.

Our previous studies with Aroclors 1242, 1248 and 1254, which are 42%, 48%, and 54% chlorine by weight, respectively, suggested that increased chlorine content of Aroclors was associated with decreased stimulation of uterine contraction frequency (Bae et al., 2001). The commercial PCB mixture Aroclor 1260 (60% chlorine by weight) was chosen for the present study. Because Aroclor 1260 consists of congeners predominantly substituted with six or more chlorines, we expected it to have comparatively low uterine stimulatory effects *in vitro*. We hypothesized that microbial dechlorination would convert the PCB mixture Aroclor 1260 to an uterotonic mixture by increasing the presence of stimulatory lower chlorinated congeners. In this study, we monitored the oscillatory contractions of uterine strips isolated from midgestation rats and exposed to PCBs *in vitro*. We compared the parent PCB mixture Aroclor 1260 with its microbially dechlorinated mixture and examined, individually and in combination, those PCB congeners that were in greatest abundance in the microbially dechlorinated mixture.

2. Materials and methods

2.1. Chemicals

The PCB mixture Aroclor 1260 and PCB congeners used in this research were purchased from Ultra Scientific (North Kingstown, RI). Dimethyl sulfoxide (DMSO) was used as a solvent for all PCB mixtures and congeners, and final exposure concentrations of DMSO did not exceed 1%.

2.2. Preparation of the microbially dechlorinated products of PCB mixtures

A microbially dechlorinated mixture, designated HR1260, was prepared by incubating Aroclor 1260 in anaerobic sediment slurries with PCB-dechlorinating microorganisms taken from Hudson River. The details of the procedure were similar to our previous reports (Bae et al., 2001), which were based on Mousa et al. (Mousa et al., 1996). After 12 months incubation with live Hudson River microorganisms, PCBs (HR1260) were extracted with hexane from the anaerobic sediment slurries. To generate a control (designated Auto1260), Aroclor 1260

was incubated with autoclaved Hudson River microorganisms and then subjected to hexane extraction using the same procedure as for HR1260. Individual PCB congeners in each sample were determined by gas chromatography (Quensen et al., 1990). The quantities were expressed as the total molar concentration of PCBs in samples and the molar percentage of congeners represented by each chromatographic peak.

Test solutions of HR1260 and Auto1260 for uterine muscle experiments were prepared by evaporating the hexane extracts and redissolving in DMSO to achieve final target concentrations. The stock solutions were analyzed in triplicate to calculate average total molar PCB concentrations, which were 73.28 mM and 93.03 mM for HR1260 and Auto1260, respectively. These stocks were diluted with DMSO as appropriate for each treatment.

2.3. Individual PCB congeners and reconstituted PCB mixture

The PCB congeners 2,2',4,4'-CB, 2,3,3',5'-CB, 2,3',4,5'-CB, 2,2',4,4',6-CB, and 2,2',4,5',6-CB were separately dissolved in DMSO to prepare 0.1 M stock solutions. Among the congeners whose abundance was statistically increased in HR1260 compared with Auto1260, a relatively weak stimulatory response was observed in the present study only with 2,2',4,5',6-CB. However, 2,2',4,4'-CB was modestly stimulatory of uterine muscle contractions in a previous study (Tsai et al., 1996). To see if these congeners exhibited synergistic effects, they were combined in DMSO to form a stock mixture of 25 mM 2,2',4,4'-CB and 10 mM 2,2',4,5',6-CB. The relative concentrations of these congeners in the reconstituted mixture reflected the relative abundance of the congeners (in mol %) present in the dechlorinated mixture HR1260.

2.4. Preparation of uterine strips

Pregnant female Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) or from the colony of the Reproductive Science Program at the University of Michigan. The preparation procedure was described in detail elsewhere (Bae et al., 2001). Briefly, pregnant rats were sacrificed on gestation day 10 by pneumothorax under anesthesia with ether. The uteri were excised and embryos were removed while in physiological salt solution (PSS) at pH 7.4 (Bae et al., 2001). From the anti-mesometrial side of the mid-portion of the uterine horns, longitudinal smooth muscle strips containing four implantation sites were cut 1 mm wide and 20 mm long.

2.5. Measurement of spontaneous oscillatory contractions

Rat uterine strips were suspended in standard muscle baths (custom made by the University of Michigan) based on the method described in our previous report (Bae et al., 2001). The muscle baths contained PSS at 36°C aerated with a mixture of 95% O₂ and 5% CO₂. Isometric contractions of the strips generated by application of a passive force of 1.0 g were monitored by a chart strip recorder. After a 40-min period in the baths, strips were exposed to 60 mM KCl to determine maximum contractile force, then rinsed and allowed an additional 4 to 6 h acclimation period for the uterine strips to establish regular spontaneous oscillatory contractions. Only strips that spontaneously generated at least 6 contractions in 10 min after acclimation were used for experiments. After the equilibration period, the 10-min interval prior to treatment was recorded as the basal period. A contraction was defined as a contraction/relaxation cycle that showed an increase of force that initiated at baseline, exceeded 15% of baseline tension, and returned to baseline. The frequency of contraction was monitored as the number of contraction/relaxation cycles in a 10-min period. The force of contraction was measured as the peak amplitude of force per contraction, averaged over the 10-min assessment period. Frequency and force of contraction were expressed as percent basal (pretreatment) values.

2.6. Measurement of chemical-induced oscillatory contractions

Uterine strips were exposed to increasing concentrations (3-70 μ M) of PCBs added to muscle bath PSS in a cumulative manner at 30-min intervals. The concentrations were selected based on previous assessments of stimulation of uterine contractions by Aroclor 1242 and Aroclor 1248 (Bae et al., 2001). Other uterine strips were exposed to DMSO as solvent controls. The last 10-min interval at each concentration was scored for analysis. All data were expressed as percent of basal frequency. Due to limited available quantities of the PCB mixtures HR1260 and Auto1260, which were extracted from cultures with live or autoclaved (dead) bacteria, respectively, these experiments had 2 to 4 uterine strips per treatment. There was a minimum of 5 uterine strips in all other treatment groups.

2.7. Statistical analysis

Contractility results are expressed as means $\pm$ standard error of the mean (SEM). Data were analyzed by two-way repeated measures analysis of variance (ANOVA) except for the HR1260 and Auto1260, which were analyzed by one-way ANOVA due to limited degrees of freedom (the quantity of available Auto1260 limited the number of replications possible). Significant effects detected by ANOVA were followed by comparisons of group means using the Student-Newman-Keuls test. All statistical analyses were performed with SigmaStat software (Jandel Corp., San Rafael, CA). For all analyses, a p-value $\leq$ 0.05 was considered statistically significant.

3. Results

3.1. Dechlorination of PCB mixtures by bacterial incubation

The congener compositions of the PCB mixtures before and after anaerobic microbial dechlorination were expressed as mole percent of total PCB content. The congener profiles are presented graphically in Figure 1 and congener identities of the gas chromatography peaks are provided in Table 1. Only the detected PCB congeners are shown. Because not all congeners could be individually separated by the gas chromatography procedure, some chromatography peaks included more than one congener.

The congener composition of Auto1260 was not altered by incubation with autoclaved inocula eluted from the Hudson River or by the extraction and sample cleanup procedures compared with Aroclor 1260 (Fig. 1A). After incubation with active (non-autoclaved) bacteria eluted from Hudson River sediment, the average total number of chlorines per biphenyl in HR1260 decreased compared with Auto1260 from 6.31 to 5.30, the average number of *ortho*-substituted chlorines decreased from 2.30 to 2.13, and the average *meta*- and *para*- substituted chlorines decreased from 4.01 to 3.17. Congeners that increased to more than 5 mol % in HR1260 (compared with Auto1260) were those associated with peak 26 (2,2',4,4'-CB) and peak 34 (2,3,3',5-CB, 2,3',4,5-CB, 2,2',4,4',6-CB, and 2,2',4,5',6-CB), which represented 23.9 and 7.6 mol %, respectively, of the total PCBs in HR1260 (Fig. 1B and 1C). Peaks 26 and 34 were not detectable in Auto1260. Congeners that decreased more than 5 mol % in HR1260 compared with Auto1260 had 6 chlorines and eluted in peak 55 (2,2',4,4',5,5'-CB) and peak 61 (2,2',3,4,4',5-CB and 2,3,3',4',5,6-CB) (Fig. 1C). Together, peaks 55 and 61 decreased from 22.6 mol % to 8.5 mol % after incubation with active bacteria.

Congeners that increased in HR1260 compared with Auto1260 were grouped by the number of chlorine atoms per biphenyl (Fig. 2). Chromatography peaks that included co-eluted congeners with different numbers of chlorines were included in each relevant group for this comparison. Congeners with four or fewer chlorines increased the greatest extent, with peaks 26 and 34 accounting for the overwhelming majority of the increase of tetrachlorobiphenyls. In Auto1260, there were no congeners with three or fewer chlorines, but congeners with one,

two or three chlorine atoms comprised 0.01, 2.2 and 11.9 mol % in HR1260. Thus, significant increases in congeners with three or fewer congeners occurred as group, even though no single congener in this group increased in relative abundance by more than 5 mol %.

3.2. Effects of Aroclor 1260 on oscillatory contractions

Initial experiments examined uterine contraction responses to Aroclor 1260 (that had not been incubated with bacteria). Aroclor 1260 (10, 30, 60, and 100 μ M), added to the muscle bath in a cumulative manner at 30-min intervals, did not significantly change the frequency of contraction (Figs. 3 and 4). The solvent control group was exposed to equivalent volumes of DMSO used to deliver Aroclor 1260, and significant changes were not observed due to the solvent (Figs. 3 and 4). The average peak force per contraction was also not significantly modified by Aroclor 1260 compared with pretreatment basal peak force of contraction (data not presented). Moreover, concentrations of Aroclor 1260 as high as 1 mM failed to stimulate uterine contraction frequency (data not shown).

3.3. Stimulation of uterine contraction by microbially dechlorinated PCB mixtures

To examine the effects of microbial dechlorination, uterine strips were exposed in muscle baths to 3, 10, 30, 50 and 70 μ M HR1260 in a cumulative manner. A significant concentration effect was observed for contraction frequency with HR1260 (ANOVA, $p < 0.001$) but not Auto1260. At 70 μ M HR1260, uterine contraction frequency was $718 \pm 134\%$ (relative to basal frequency), which was significantly higher compared with contraction frequencies of uterine strips exposed to lower concentrations of HR1260 (3, 10, 30 and 50 μ M, $p < 0.001$; Fig. 5). In contrast, uterine strips exposed to 3, 10, 30, 50 and 70 μ M Auto1260 (PCB mixture extracted from cultures of autoclaved bacteria incubated with Aroclor 1260) displayed no significant changes in contraction frequency (Fig. 5) or force (data not shown), similar to the results with Aroclor 1260. Neither Aroclor 1260 nor HR1260 had significant effects on contraction force (not shown).

3.4. Stimulation of uterine contraction by individual PCB congeners

Because of the significant increase of chromatography peaks 26 and 34 in the dechlorinated PCB mixture (HR1260 in Fig. 1), the tetra- and pentachlorobiphenyls that possibly eluted in peaks 26 and 34 were examined individually in an attempt to identify the particular congeners responsible for the stimulatory activity of HR1260 on uterine contractions. Exposure to 60 and 100 μ M 2,2',4,5',6-CB increased uterine contraction frequency compared with solvent control strips (Fig. 6; $p < 0.05$), although the magnitude of increase was much less than that obtained with HR1260 (compare with Fig. 5). The frequency of contraction was not significantly altered for uterine strips exposed in muscle baths to 10, 30, 60 or 100 μ M of 2,2',4,4'-CB, 2,3,3',5-CB, 2,3',4,5-CB, or 2,2',4,4',6-CB (Fig. 6). The individual congeners did not significantly alter contraction force (not shown).

3.5. Stimulation of uterine contraction by a reconstituted PCB mixture

Because a relatively weak stimulatory response was observed with 2,2',4,5',6-CB and 2,2',4,4'-CB was modestly stimulatory of uterine muscle contractions in a previous study (Tsai et al., 1996), these compounds were mixed in the ratio found in the dechlorinated PCB mixture HR1260. The reconstituted PCB mixture stimulated contraction frequency relative to controls (ANOVA treatment effect, $p = 0.004$; Fig. 7), with no significant effect on contraction force (not shown). At 60 and 100 μ M of the reconstituted mixture, uterine contraction frequencies were $123.2 \pm 13.2\%$ and $111.4 \pm 5.3\%$, significantly increased compared with the solvent controls exposed to DMSO used to deliver the PCBs at each concentration (Fig. 7; $p < 0.05$). However, the response to the reconstituted mixture was much less than the response observed with HR1260 (compare with Fig. 5).

4. Discussion

The results of the present study indicate that microbial dechlorination converted the commercial PCB mixture Aroclor 1260 from a mixture lacking significant acute activity on the spontaneous contraction of pregnant rat uterus to a mixture capable of profoundly stimulating the frequency of uterine contraction. Microorganisms eluted from the PCB-contaminated Hudson River effectively dechlorinated Aroclor 1260, removing on average one chlorine per biphenyl molecule; chlorines were removed primarily from the *meta*- and *para*-positions of the biphenyl. This microbial dechlorination shifted the composition of the PCB mixture from one dominated by heavily chlorinated congeners such as 2,2',3,4,4',5'-CB and 2,3,3',4',5,6-CB to a mixture characterized by an increased abundance of congeners with four or fewer chlorines.

Aroclor 1260 did not increase uterine muscle contraction frequency up to 1 mM (data not shown). Our previous studies showed that Aroclor 1248 and Aroclor 1242 increase the frequency of uterine contractions at 100 μ M, and Aroclor 1254 increases the frequency of uterine contractions at 1 mM (Bae et al., 2001). Aroclor 1242, 1248, 1254 and 1260 contain 42%, 48%, 54% and 60% chlorine by weight, respectively. Consequently, the lack of a stimulatory effect of Aroclor 1260, as well as the ability of microbially dechlorinated Aroclor 1260 to stimulate uterine contractions, is consistent with our previous finding (Bae et al., 2001) that uterotonic activity of PCB mixtures is inversely related to chlorine content, as assessed in pregnant rat uterus *in vitro*.

The stimulatory effects observed with the microbially dechlorinated mixture HR1260 could not be fully explained by the uterotonic activities of those few individual congeners that increased to the greatest extent because of dechlorination. Although 2,2',4,4'-CB increased to 24 mol % after microbial dechlorination, no significant increase in uterine contraction was observed in strips exposed to 2,2',4,4'-CB at the concentrations used in the present study (10 to 100 μ M). This result did not agree with our previous study, in which we reported an increase of contraction frequency at 30 μ M and 100 μ M of 2,2',4,4'-CB (Tsai et al., 1996). Given the discrepancy of results, the effects of 2,2',4,4'-CB on uterine contractions frequency were examined at the higher concentrations of 500 μ M and 700 μ M, and a statistically significantly increased frequency was observed for uterine strips exposed to 2,2',4,4'-CB at these higher concentrations (data not shown). The reason for the decreased responsiveness of uterine strips to 2,2',4,4'-CB in the present study compared to that of Tsai et al. (Tsai et al., 1996) is not certain, but may be related to differences between animals, tissue preparations or muscle bath conditions.

Among the congeners that possibly co-eluted in a chromatography peak that increased to 7.6 mol % after microbial dechlorination, 2,2',4,5',6-CB was the only one that stimulated contraction frequency. However, at comparable concentrations, this congener increased the frequency of contraction only one-sixth of that observed with HR1260. Therefore, although two chromatography peaks increased more than 5 mol % after microbial dechlorination and accounted for 31 mol % of HR1260, the congeners that possibly eluted in those peaks did not demonstrate sufficient individual stimulatory activity to account for the increased frequency of contractions produced by HR1260. Furthermore, a mixture of 2,2',4,4'-CB and 2,2',4,5',6-CB, combined in molar ratios found in HR1260, stimulated the frequency of contraction to a much less extent than was observed in response to HR1260.

Because 2,2',3,5',6-pentachlorobiphenyl enhances binding of ryanodine to its receptor (Wong et al., 1997a; Wong et al., 1997b) and is similar in structure to 2,2',4,5',6-CB, found in HR1260, ryanodine receptor activation might play a role in 2,2',4,5',6-CB stimulation of uterine contraction frequency. However, the relatively weak stimulation of uterine contraction

frequency by 2,2',4,5',6-CB alone and in combination with 2,2',4,4'-CB suggests that ryanodine receptor activation by 2,2',4,5',6,-CB cannot alone explain the increased uterotonic activity of the microbially dechlorinated PCB mixture HR1260. The residue of lipids from microorganisms, sulfur, and break-down compounds from the biphenyl rings could not account for the stimulatory effect of HR1260, either, because they were removed from the solvent extract during the clean-up process. Apparently, some more intricate effect on uterine contraction frequency is produced by the more complex mixture of congeners in HR1260 compared to the simple combination of the few most abundant dechlorinated congeners in HR1260.

Reductive anaerobic dechlorination of PCBs is a common microbial environmental transformation that preferentially removes chlorines from the *meta* and *para* positions while leaving the biphenyl rings intact (Quensen et al., 1990). In riverbed sediments and in the laboratory, microbial dechlorinations of commercial PCB mixtures produce mixtures with decreased percentages of the more heavily chlorinated congeners (with five or more chlorines) and increased percentages of *ortho*-substituted lesser chlorinated congeners (Tiedje et al., 1993), as observed in the present study. In contrast to the remarkably increased uterotonic activity observed with microbial dechlorination previously (Bae et al., 2001) and in the present study, microbial dechlorination of commercial PCB mixtures decreases aryl hydrocarbon receptor-mediated activity in rat hepatoma cells (Quensen et al., 1998) and decreases inhibition of mouse gamete fertilization *in vitro* (Mousa et al., 1996). The latter findings were attributed to a decrease in the relative abundance of coplanar congeners lacking *ortho* chlorines.

When the individual PCB congeners tested in this study (2,3,3',5-CB, 2,3',4,5-CB, 2,2',4,4',6-CB and 2,2',4,5',6-CB) are excluded, the majority of the remaining congeners that increased in relative abundance are *ortho*-substituted PCBs with three or fewer chlorines, which as a group increased to 14.1 mol % in HR1260. The inability of microbial dechlorination to modify activity of commercial PCB mixtures on insulin secretion, neutrophil activation and splenocyte proliferation has been attributed to the presence of *ortho*-substituted PCB congeners with three or fewer chlorines (Ganey et al., 2000; Smithwick et al., 2004). Consistent with the latter report, structure-activity studies in our laboratory show that *ortho*-substituted di- and trichlorobiphenyls elicited increased uterine contraction frequencies of the magnitude similar to that observed with HR1260 (in preparation). We suggest that the uterotonic activity of HR1260 may be due to the increased relative abundance of these lesser chlorinated *ortho*-substituted PCBs after microbial dechlorination, even though the abundance of no one congener in this group exceeded 5 mol % of the total.

Because of limited quantities of PCB mixtures available from the bacterial cultures, the PCB mixtures were added to muscle baths in a cumulative manner. However, the cumulative concentration-response design may not reveal the maximal response at any particular concentration because the uterine response may change with increased duration of exposure. The concentrations of PCB congeners and mixtures that stimulated uterine contraction frequency *in vitro* in the present study and in previously reported studies (Bae et al., 2001; Loch-Caruso, 2002; Tsai et al., 1998; Tsai et al., 1996) are much greater than PCB concentrations reported in human tissue samples and are higher than concentrations in the environment. Nonetheless, the concentrations used in the present study are similar to those required to stimulate insulin secretion, neutrophil activation and splenocyte proliferation and to inhibit mouse gamete fertilization *in vitro* (Mousa et al., 1996).

In summary, incubation of the PCB mixture Aroclor 1260 with microorganisms isolated from sediments of the PCB-contaminated Hudson River decreased chlorine content in the PCB mixture. The microbially dechlorinated PCB mixture HR1260 contained a greater abundance of biphenyls with four or fewer chlorines compared to the parent mixture Aroclor 1260. As a

result of the dechlorination, the non-uterotonic PCB mixture Aroclor 1260 was converted to the uterotonic PCB mixture HR1260 that greatly increased the frequency of contraction in pregnant rat uterus *in vitro*. However, the stimulatory effects of HR1260 were not fully explained by those few specific congeners whose relative abundance individually increased most in the mixture after microbial degradation. Although not directly tested in the present study, it is suggested that *ortho*-substituted congeners with three or fewer chlorines contribute to the uterotonic activity of the microbially dechlorinated HR1260 mixture because the relative abundance of these lightly chlorinated congeners significantly increased as a group.

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Figure 1.

The gas chromatographic profiles of: (A) Auto1260, the essentially unaltered PCB mixture extracted after incubation of Aroclor 1260 with autoclaved bacteria; (B) HR1260, the dechlorinated product mixture obtained after anaerobic incubation of Aroclor 1260 in sediment slurries with microorganisms eluted from the upper Hudson River; and (C) differences in abundance of PCB congeners in HR1260 compared with Auto1260. Data are expressed as mole percent. Peak 26 corresponds to 2,2',4,4'-tetrachlorobiphenyl, and peak 34 may include the co-eluting congeners 2,3,3',5-tetrachlorobiphenyl, 2,3',4,5-tetrachlorobiphenyl, 2,2',4,4',6-pentachlorobiphenyl and 2,2',4,5',6-pentachlorobiphenyl.

Figure 2.

The increase in relative abundance of congeners in HR1260 compared with Auto1260 grouped by the number of chlorine atoms per biphenyl. The chromatography peaks from Fig. 1 that increased more than 5 mol % in HR1260 compared with Auto1260 (peaks 26 and 34) possibly included congeners with four or five chlorines and are shown in a separate column. Among mono-substituted congeners, only 4-CB increased in HR1260 compared with Auto1260 (0.01 mol %) and this increase was too small to be depicted graphically. Chromatography peaks that included co-eluted congeners with different numbers of chlorines were included in each relevant group.

Figure 3.

Representative polygraph tracings of uterine strips exposed *in vitro* to increasing concentrations of Aroclor 1260, Auto1260, or HR1260. Control strips were exposed to DMSO concentrations used to deliver PCB mixtures. Compounds were added to the muscle baths in a cumulative manner at 30-min intervals. Polygraph tracings were taken from the last 10 min of exposure at each concentration.

Figure 4.

Frequency of contraction of uterine strips exposed *in vitro* to increasing concentrations of Aroclor 1260 added in a cumulative manner at 30-min intervals. Control strips were exposed to DMSO concentrations used to deliver Aroclor 1260. Contraction frequency was determined for the last 10 min of each exposure and expressed as percent of basal contraction frequency. Basal values were obtained from the 10-min period immediately prior to the addition of PCB or DMSO. Data are expressed as mean $\pm$ standard error of the mean. No statistically significant effects of treatment or concentration were observed (n=6).

Figure 5.

Effects of Auto1260 and HR1260 on uterine contraction. Auto1260 was a control PCB mixture extracted after incubation with autoclaved (dead) microorganisms and HR1260 was generated by incubation with active microorganisms from Hudson River sediment. Contraction frequency was determined for the last 10 min of each exposure and expressed as percent of basal contraction frequency. Basal values were obtained from the 10-min period immediately prior to the addition of PCB. A significant concentration effect was observed with HR1260 (ANOVA, $p < 0.001$) but not Auto1260. Data are expressed as mean $\pm$ standard error of the mean. The asterisk represents a statistically significant difference from all other concentrations of HR1260 (Student-Newman-Kuels, $P < 0.05$). There were 2 and 4 strips in the Auto1260 and HR1260 groups, respectively.

Figure 6.

The frequency of contraction of uterine strips exposed *in vitro* to PCB congeners present in chromatography peaks 26 and 34 (see Fig. 1): 2,2',4,4'-tetrachlorobiphenyl, 2,3,3',5-tetrachlorobiphenyl, 2,3',4,5-tetrachlorobiphenyl, 2,2',4,4',6-pentachlorobiphenyl, and 2,2',4,5',6-pentachlorobiphenyl. Control strips were exposed to DMSO concentrations used to deliver PCBs. Contraction frequency was determined for the last 10 min of each exposure and expressed as percent of basal contraction frequency. Basal values were obtained from the 10-min period immediately prior to the addition of PCB or DMSO. Data are expressed as mean $\pm$ standard error of the mean. Asterisks represent statistically significant difference from controls (Student-Newman-Keuls, $P < 0.05$). There were 5-8 strips per group.

Figure 7.

Effects of a reconstituted PCB mixture on uterine contraction. Uterine strips were exposed *in vitro* to a reconstituted PCB mixture consisting of 2,2',4,4'-tetrachlorobiphenyl and 2,2',4,5',6-pentachlorobiphenyl combined in a molar ratio of 2.5:1. Control strips were exposed to DMSO concentrations used to deliver the PCB mixture. Contraction frequency was determined for the last 10 min of each exposure and expressed as percent of basal contraction frequency. Basal values were obtained from the 10-min period immediately prior to the addition of PCB or DMSO. The error bars represent the standard error. Asterisks indicate statistically significant differences from controls (Student-Newman-Keuls, $P < 0.05$). There were 12-15 uterine strips per group.

Table 1

Congener identities of chromatography peaks

Peak No.	Congener	Peak No.	Congener
4	2,2'-/2,6-CB ^a	47	2,2',3,4,4'-CB
5	2,4-/2,5-CB	48	2,2',3,3',6,6',-CB
6	2,3'-CB	49	3,3',4,4'-/2,3,3',4',6-CB
7	2,4'-/2,3-CB	50	2,2',3,5,5',6-CB
8	2,2',6-CB	51	2,2',3,3',5,6'-/2',3,4,5,5'-/2,2',3,4,5',6-CB
9	3,4-/3,4'-CB	52	2,3,4,4',5-/2,2',3,4',5',6-/2,3,3',4,5-CB
10	2,2',5-/4,4'-CB	53	2,2',3,3',5,6-/2,2',3,4,5,6-/2,3,4,4',5-CB
11	2,2',4-CB	54	2,2',3,4',5,5'-/2,3,3',4,5',6-CB
12	2,3',3-/2,3,6-CB	55	2,2',4,4',5,5'-CB
13	2,2',3-/2,4',6-CB	56	2,2',3,3',4,6'-/2,3,3',4,4'-CB
14	2',3,5- (2,2',6,6'-)CB	57	2,2',3,4,5,5'-CB
15	2,4,5-CB	58	2,2',3,3',5,6,6'-CB
16	2,3',5-CB	59	2,2',3,4,4',5-CB
17	2,3',4-CB	60	2,2',3,3',4,6,6'-CB
18	2,4',5-CB	61	2,2',3,4,4',5'-/2,3,3',4',5,6-CB
19	2,4,4'- (2,2',4,6-)CB	62	2,3,3',4,4',6-CB
20	2',3,4-/2,3,4-/2,3,3'-/2,2',5,6'-CB	63	2,2',3,3',5,5',6-CB
21	2,4',3- (2,2',4,6'-)CB	64	2,2',3,3',4,5',6-CB
22	2,2',3,6-CB	65	2,2',3,4',5,5',6-/2,2',3,4,4',5,6-CB
23	2,2',3,6'-CB	66	2,2',3,4,4',5',6-CB
24	2,2',5,5'-/2,3',5',6-CB	67	2,3',4,4',5,5'-CB
25	2,2',4,5'-CB	68	2,2',3,4,5,5',6-CB
26	2,2',4,4'-CB	69	2,2',3,3',4,5,6'-/2,2',3,4,4',5,6-CB
28	3,3',4-CB	70	2,2',3,3',4',5,6-CB
29	2,2',3,5'-CB	71	2,2',3,3',4,4',6-/2,3,3',4,4',5-/2,2',3,3',5,5',6,6-CB
30	2,2',3,4'-/2,3,3',6-/3,4,4'-CB	72	2,2',3,3',4,5,6-/2,2',3,3',4,5',6,6'-/2,2',3,4,4',5,6,6'-CB
31	2,3',4',6-/2,2',3,4-/2,3,4',6-/2,3',5,5'-CB	73	2,2',3,3',4,5,5'-/2,3,3',4,5,5',6-CB
32	2,2',3,6,6'-CB	74	2,2',3,4,4',5,5'-CB
33	2,2',3,3'-CB	75	2,3,3',4',5,5',6-CB
34	2,3,3',5-/2,3',4,5-/2,2',4,4',6-/2,2',4,5',6-CB	76	2,3,3',4,4',5',6-CB
35	2,3,3',5-,2,3,4',5-CB	77	2,2',3,3',4,5,6,6'-CB
36	2,4,4',5- (2,2',3,5,6'-)CB	78	2,2',3,3',4,4',5-CB
37	2,3',4',5-/2',3,4,5-CB	79	2,3,3',4,4',5,6-CB
38	2,2',3,5',6-/2,2',4,5,6'-/2,3,4,4'-CB	80	2,2',3,3',4',5,5',6-CB
39	2,3,3',4-/2,2',3,4',6-CB	81	2,2',3,3',4,4',5,6'-/2,2',3,4,4',5,5',6-CB
40	2,2',4,4',6,6'- (Surrogate)CB	82	2,3,3',4,4',5,5'-CB
41	2,3,3',4'-/2,3,4,4'-CB	83	2,2',3,3',4,4',5,6-CB
42	2,2',4,5,5'-/2,2',3,4',5-CB	84	2,2',3,3',4,5,5',6,6'-CB
43	2,2',4,4',5-CB	85	2,2',3,3',4,4',5,5'-CB
45	2,2',3',4,5-/2,2',3,4,5- (2,2',3,5,6,6'-)CB	86	2,3,3',4,4',5,5',6-CB

Peak No.	Congener	Peak No.	Congener
46	2,2',3,4,5'-/2,3,4,4',6-/2,3,3',5,5'-CB	87	2,2',3,3',4,4',5,5',6-CB

^aCB, chlorobiphenyl