Stimulation of Contraction of Pregnant Rat Uterus *in Vitro* by Non-Dechlorinated and Microbially Dechlorinated Mixtures of Polychlorinated Biphenyls

Jeehyeon Bae,[1] Mahmoud A. Mousa,[2] John F. Quensen, III,[2] Stephen A. Boyd,[2] and Rita Loch-Caruso[1]

[1]Toxicology Program, Department of Environmental Health Sciences, The University of Michigan, Ann Arbor, Michigan, USA; [2]Department of Crop and Soil Sciences and Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan, USA

A previous study of six polychlorinated biphenyl (PCB) congeners showed that PCBs with four or fewer chlorines and *ortho* substitution stimulate uterine contraction frequency *in vitro*, whereas congeners with a greater number of chlorines or non-*ortho* substitution are inactive *in vitro*. We tested the hypothesis that PCB mixtures stimulate uterine contractions in a manner inversely related to the degree of chlorination and the presence of chlorines in the *ortho*- position of the biphenyl constituents of the mixtures. Uterine strips from pregnant rats were suspended in standard muscle baths and analyzed for changes in isometric contractions in response to *in vitro* exposure to commercial PCB mixtures (Aroclors) and their dechlorinated products after microbial degradation. The PCB mixtures Aroclor 1242, 1248, and 1254 significantly stimulated uterine contraction frequency, and the least chlorinated mixture, Aroclor 1242, was the most potent stimulant. Microbes from Hudson River sediment dechlorinated Aroclor 1242 and Aroclor 1254 under reducing conditions to produce mixtures with an increased proportion of *ortho*-substituted congeners with one or two chlorine substitutions. The PCB mixtures that had undergone microbial reductive dechlorination stimulated uterine contraction frequency to a significantly greater extent than the parent mixtures. These results show that increased uterotonic activity was associated with decreased chlorination and increased *ortho* substitution of the biphenyl constituents of the mixtures. *Key words*: Aroclor, dechlorination, muscle contraction, polychlorinated biphenyls, pregnancy, uterus. *Environ Health Perspect* 109:275–282 (2001). [Online 1 March 2001] *http://ehpnet1.niehs.nih.gov/docs/2001/109p275-282bae/abstract.html*

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants previously used in transformers, in the manufacture of paints, and for various other industrial purposes. PCBs were used and marketed as mixtures of PCB congeners under trade names such as Aroclor. Even though PCB production in the United States was banned in 1977, PCB production in other countries continued into the 1990s (*1*). Because of their environmental persistence and ability to bioaccumulate, PCBs are present in human and wildlife tissues (*2*).

Several epidemiologic studies suggest that PCBs may interfere with the ability to maintain a pregnancy to term. These studies report increased blood or blood serum PCB levels in women who delivered prematurely compared with women who went to term (*3–5*) and decreased gestation length in women exposed to PCBs occupationally (*6,7*) or by consumption of contaminated food (*8–10*). A positive association between PCB exposure and spontaneous abortion in women was also reported (*4*). However, other epidemiologic studies failed to detect a significant relationship between premature birth or spontaneous abortion and exposure to PCBs (*11–14*).

Although not widely studied, reports indicate that PCBs modify parturition in animals. Reported PCB effects on parturition in laboratory animals include increased variation of gestation length and incidence of difficult labor in guinea pigs (*15*), increased gestation length in rats (*16–18*), and spontaneous abortion in monkeys (at doses toxic to the mother) (*19,20*). Elevated tissue concentrations of PCBs were reported in California sea lions that delivered their pups prematurely (*21*).

Parturition is a complex process that requires distinct as well as interdependent physiologic activities. Timely and effective uterine contraction is a critical component of parturition (*22*). In pregnant women, increased frequency of synchronized uterine contractions before term is associated with preterm labor (*22–25*). Stimulation of uterine contraction is a plausible mechanism whereby a chemical could induce preterm labor, decrease gestation length, or induce abortion. Because PCBs distribute into the lipids of the uterus to a significant extent during pregnancy in women (*26*) and in laboratory animals (*27*), the pregnant uterus may be a target of PCB action.

Previous work in our laboratory showed that several PCB congeners with *ortho*-chlorine substitutions and four or fewer chlorines acutely increased uterine contraction frequency, whereas coplanar congeners or congeners with greater numbers of chlorines were inactive (*28*). Because individual PCB congeners elicit different effects on the uterus, yet most contamination occurs as mixtures, it was of interest to examine the uterine muscle response to PCB mixtures.

We hypothesized that PCB mixtures directly stimulate uterine oscillatory contractions in a manner inversely related to the degree of chlorination and the presence of chlorines in the *ortho* position of the biphenyl constituents of the mixtures. The commercial PCB mixtures Aroclor 1242, Aroclor 1248, and Aroclor 1254 were chosen for this study. The last two digits of the Aroclor name indicate the percent chlorine content by weight (e.g., Aroclor 1242 contains 42% chlorine). These once commonly used mixtures typically contain 60–90 of the 209 possible PCB congeners (*29*). Compared with Aroclors 1248 and 1254, Aroclor 1242 has a greater proportion of *ortho*-substituted congeners with three or fewer chlorines (*30*). Also, compared with Aroclor 1254, Aroclors 1242 and 1248 have greater proportions of *ortho*-substituted congeners with four or fewer chlorines (*30*). Because reductive dechlorination of PCBs is a common environmental transformation in anaerobic sediments (*31–33*), we hypothesized further that microbial dechlorination of Aroclors would produce PCB mixtures with increased uterotonic activity. To test this latter hypothesis, we evaluated Aroclor 1242 and Aroclor 1254 that had undergone reductive dechlorination (designated as HR1242 and HR1254, respectively) by microbes obtained from sediments of the PCB-contaminated Hudson River. We applied standardized muscle bath procedures for working with midgestation uteri to test these hypotheses, monitoring the oscillatory contractions of uterine strips exposed to PCBs *in vitro*.

Materials and Methods

Chemicals. Aroclors 1242, 1248, and 1254 were purchased from Ultra Scientific (North

Address correspondence to R. Loch-Caruso, Toxicology Program, Department of Environmental Health Sciences, The University of Michigan, 1420 Washington Heights, Ann Arbor, MI 48109-2029 USA. Telephone: (734) 936-1256. Fax: (734) 647-9770. E-mail: rlc@umich.edu

We thank C. Harris for providing uterine tissues.

This project was supported by NIH grant P42-ES04911 to S.A. Boyd, R. Loch-Caruso, and J.F. Quensen, III. Additional support was provided by the Laboratory Animal Core of the Center for the Study of Reproduction at the University of Michigan (NIH P30-HD18258).

Received 29 November 1999; accepted 24 October 2000.

Kingstown, RI) or were gifts from Monsanto to the Pesticide Research Center at Michigan State University. All of the PCB mixtures were dissolved in DMSO, and final exposure concentrations of DMSO did not exceed 1%. The congener compositions of the Aroclors were determined by gas chromatography as described in the next section.

Preparation of the microbial dechlorination products of PCB mixtures. The commercial PCB mixtures Aroclor 1242 and Aroclor 1254 were incubated in anaerobic sediment slurries inoculated with PCB-dechlorinating microorganisms eluted from PCB-contaminated upper Hudson River (HR) sediments, using methods previously described (*34*). Briefly, the slurries were prepared in an aerobic glove box by mixing 600 g air-dried and sieved (2 mm) non–PCB-contaminated Red Cedar River (Okemos, MI) sediment and 600 mL of revised anaerobic mineral medium (RAMM) in 1-L bottles. The bottles were then sealed, incubated until methane was detected in the headspace (about 2 weeks), and autoclaved. An acetone solution (20%) of Aroclor 1242 or 1254 was then added to each bottle to a concentration of 600 µg/g sediment dry weight (parts per million). An inoculum of PCB-dechlorinating microorganisms was also prepared under anaerobic conditions by vigorously mixing 1 L of wet upper Hudson River sediment with 1 L of RAMM in a tightly stoppered Erlenmeyer flask, allowing the sediment to settle, and decanting the supernatant. We used this supernatant to inoculate the sediment slurries, which were then capped and sealed with tape in the anaerobic chamber, mixed thoroughly, and incubated at room temperature. We monitored the dechlorination process periodically by withdrawing, extracting, and analyzing samples for changes in PCB congener profile. Control sediment slurries containing autoclaved (dead) microorganisms were incubated with Aroclor 1242 or 1254 to produce control PCB mixtures (Auto1242 and Auto1254, respectively), as described previously. A control containing active microorganisms and sediments with no PCBs was also included. No PCBs were detected in the latter control, and this extract was not tested further. The incubations were terminated after 20 months.

After the incubation period, the PCBs were extracted from the sediment slurries. First the liquid contents of each bottle were decanted into a 1-L separatory funnel. PCBs were then extracted from the sediments three times with 500-mL portions of acetone and three times with 500-mL portions of hexane:acetone (9:1, v:v). The sediments were shaken vigorously with each portion of extraction solvent for 1 hr before the solvent was decanted into the separatory funnel. The volume of extraction solvents in the funnel was reduced by evaporation under N_2 as needed to accommodate subsequent portions of extract, and upon addition of the first portion of hexane:acetone to each funnel, the lower aqueous phase with acetone was drained from the funnel. After the extraction was completed, the remaining acetone was back-extracted with 2% NaCl in deionized water. The remaining hexane solution was treated five times with 25-mL portions of concentrated sulfuric acid, washed three times with 50-mL portions of 2% NaCl, dried over anhydrous sodium sulfate, and passed through 50 mL of Florisil and acid-rinsed copper powder contained in a 100-mL burette.

We determined the total molar PCB concentrations and the identities and concentrations of the individual congeners in each sample by gas chromatography (*35*). Test solutions for the uterine muscle experiments were prepared by evaporating the hexane and redissolving the PCBs in DMSO to similar final target concentrations. We analyzed these stock solutions in triplicate to calculate the average total molar concentrations, which ranged from 72 mM to 90 mM.

Preparation of uterine strips. Pregnant (gestation day 10) rats were obtained from Harlan (Indianapolis, IN) or from the colony of the Reproductive Science Program at the University of Michigan. Nulliparous female rats weighing 180–220 g were mated between 60 and 90 days of age. The animals were housed at 24 ± 1°C under a 14-hr light schedule. Pregnant rats were anesthetized with ether followed by exsanguination, a protocol required by collaborators with whom we shared tissue. After isolating uteri, embryos and fat were removed. Longitudinal uterine strips 1 mm wide by 20 mm long were cut from the anti-mesometrial side of the mid-portion of horns which contained four implantation sites.

Measurement of spontaneous oscillatory contractions. The uterine strips were suspended in standard muscle baths that contained physiologic salt solution (PSS) composed of 116 mM NaCl, 4.6 mM KCl, 1.16 mM $NaH_2PO_4 \cdot H_2O$, 1.16 mM $MgSO_4 \cdot 7H_2O$, 21.9 mM $NaHCO_3$, 1.8 mM $CaCl_2 \cdot 2H_2O$, 11.6 mM dextrose, and 0.03 mM $CaNa_2EDTA$ at pH 7.4. The water-jacketed bath was maintained at 36°C and aerated with a mixture of 95% O_2 and 5% CO_2. Each uterine strip was tied with surgical silk to a stationary post at one end and to an isometric force transducer at the other end. We measured spontaneous oscillatory contractions as described previously (*36*), with modifications. Briefly, isometric contractions of strips were monitored under constant passive force of 1.0 g. After a 40-min equilibration period, strips were challenged with 60 mM KCl to determine viability and maximum KCl-induced contraction force. After rinsing out the KCl, strips were allowed to equilibrate for an additional 2–5 hr to establish regular spontaneous oscillatory contractions. We measured contractions by frequency (number of contraction/relaxation cycles in a 10-min period) because this was the most prominent

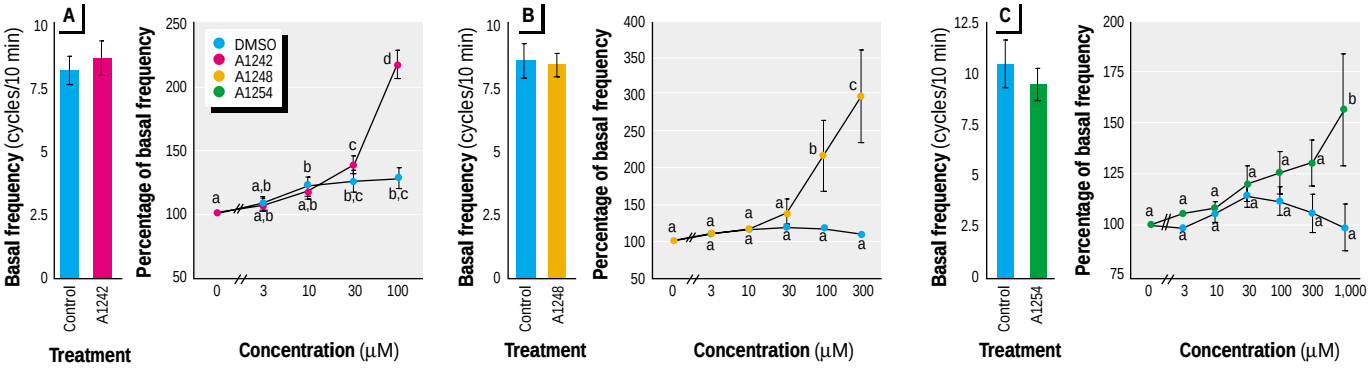

Figure 1. Concentration-dependent effects on the frequency of contraction of uterine strips exposed *in vitro* to (*A*) Aroclor 1242 (A1242), (*B*) Aroclor 1248 (A1248), and (*C*) Aroclor 1254 (A1254). Left, average basal contraction frequency of uterine strips before treatment with DMSO (solvent control) or Aroclor. Right, contraction frequency response to Aroclors. Values shown are mean ± SEM of 8–10 uterine strips. If no error bar is shown, the SEM is smaller than the size of the symbol. Means sharing the same letter are not significantly different ($p \leq 0.05$).

parameter affected by Aroclors. The frequency during the 10-min segment after equilibration and before any treatment was termed "basal frequency."

Measurement of chemical-induced oscillatory contractions. In some experiments, cumulative concentration–response curves were generated by exposing uterine strips to increasing concentrations of PCB mixtures added to the muscle bath in a cumulative manner at 20-min intervals. The range spanned from concentrations that elicited no effect to concentrations that elicited the maximal effect measurable. Because time is a possible confounding variable in cumulative concentration experiments, other strips were exposed to a single concentration of Aroclor 1242 for 60 min. To examine reversibility, we exposed strips to different concentrations of Aroclor 1242 for 60 min, then rinsed them three times with PSS and monitored them for up to 4 hr. In each experiment, solvent control uterine strips were exposed to equivalent concentrations of DMSO added in a similar manner as the PCBs. All data were normalized with respect to basal frequency of contraction and expressed as percent of basal frequency. We included 8–11 uterine strips in each treatment group exposed to neat (parent) Aroclors. However, quantities of the PCB mixtures extracted from cultures with live or autoclaved bacteria limited these experiments to 2–6 uterine strips per treatment.

Statistical analysis. Results are expressed as mean ± SEM. We analyzed contractility data by two-way repeated measure analysis of variance (ANOVA), except for the HR1254 and Auto1254 data, which were analyzed by one-way ANOVA because of limited degrees of freedom (the quantity of Auto1254 available 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 < 0.05 was considered statistically significant.

Results

Concentration-dependent effects of Aroclors on oscillatory contractions. Each Aroclor was examined in separate experiments with distinct solvent (DMSO) control groups. The most prominent and significant effect of these PCB mixtures on uterine contraction was to increase the frequency of oscillatory contractions. Although contractile force decreased somewhat at the highest concentrations of Aroclors tested (data not shown), this effect appeared to be secondary to profound increases of frequency.

Compared with pretreatment (0 µM Aroclor 1242), exposure to 10, 30, or 100 µM Aroclor 1242 significantly stimulated contractions to 116.6 ± 6.1, 136.5 ± 6.4, and 214.9 ± 10.6%, respectively (Figure 1A; $p < 0.05$). In comparison with solvent controls, however, contraction frequency was significantly increased only at 100 µM Aroclor 1242 (Figure 1A; $p < 0.05$). In the solvent control strips exposed to DMSO concentrations used to deliver 30 and 100 mM Aroclor 1242, the frequency of contraction also increased significantly, albeit modestly, compared with pretreatment (0 µM DMSO; Figure 1A; $p < 0.05$). As a consequence, the statistically significant increases detected at 10 mM and 30 µM Aroclor 1242 in comparison with 0 µM Aroclor 1242 should be interpreted with caution.

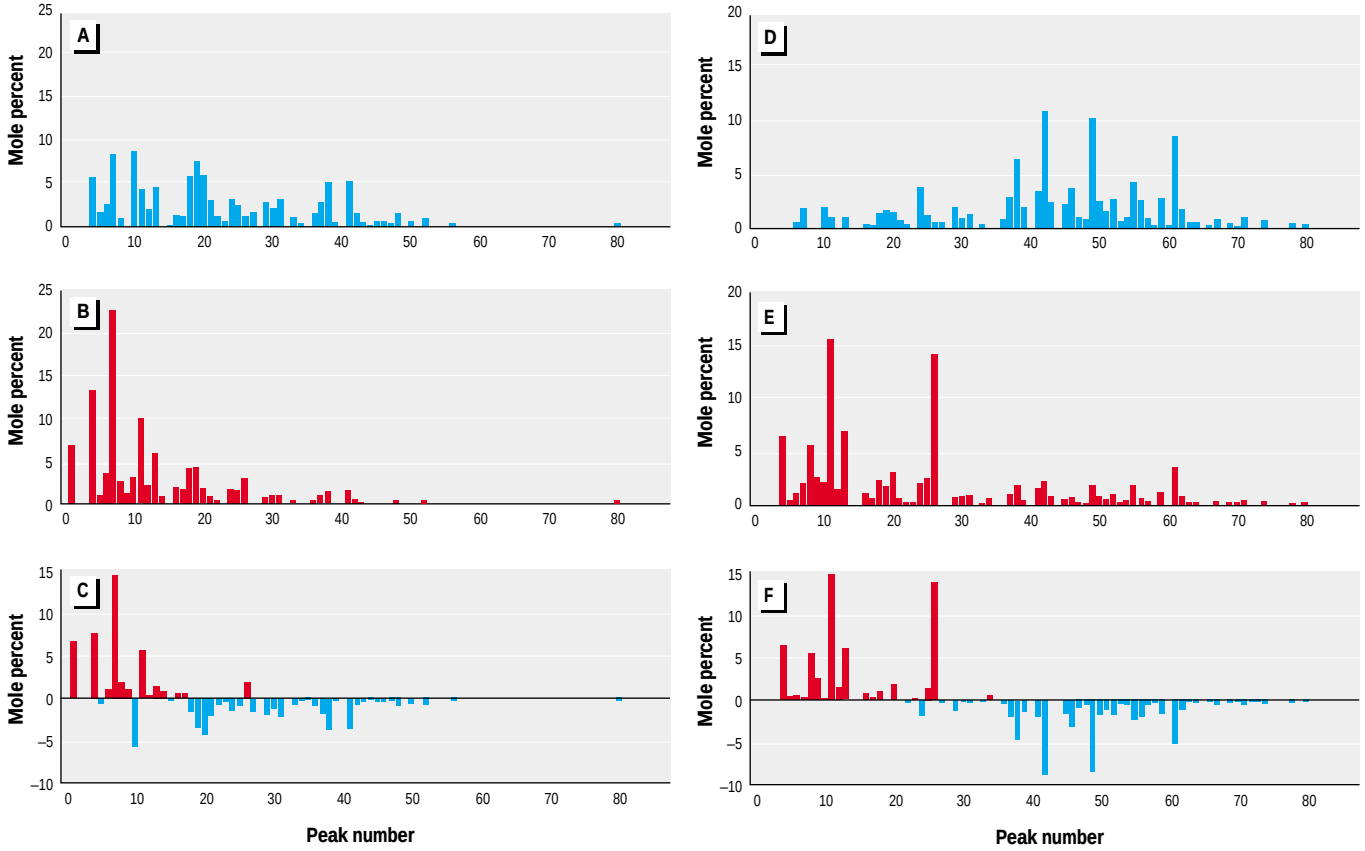

Figure 2. The gas chromatographic profiles of (*A*) Auto1242, the PCB mixture extracted after incubation of Aroclor 1242 with autoclaved bacteria (control for nonmicrobial alteration of PCBs); (*B*) HR1242, the dechlorinated product mixture obtained after incubation of Aroclor 1242 with microorganisms; and (*C*) the differences in molar percent of PCB congeners in HR1242 compared with Auto1242. The gas chromatographic profiles of (*D*) Auto1254, the PCB mixture extracted after incubation of Aroclor 1254 with autoclaved bacteria (control for nonmicrobial alteration of PCBs); (*E*) HR1254, the dechlorinated product mixture after incubation of Aroclor 1254 with the Hudson River microorganisms; and (*F*) the differences in molar percent of PCB congeners in HR1254 compared with Auto1254. The PCB congener correspondence to each peak is given in Table 1.

Contraction frequency could not be quantified at 300 µM Aroclor 1242 because this exposure produced contractions with sustained increased basal tension.

Exposure of uterine strips to Aroclor 1248 at 100 mM and 300 mM increased contraction frequency to 213.5 ± 48.0% and 294.3 + 64.0%, respectively (Figure 1B). These stimulations were significant as compared with both pretreatment (0 µM Aroclor 1248) and solvent controls ($p < 0.05$). Aroclor 1254-treated strips showed a significant stimulation of contraction only at 1,000 µM (155.4 ± 27.2%) relative to pretreatment (0 mM Aroclor 1254) and solvent controls (Figure 1C; $p < 0.05$).

Dechlorination of PCB mixtures by bacterial incubation. Gas chromatography identified and quantified PCB congeners extracted after incubation of Aroclor 1242 (Figure 2A–C) or Aroclor 1254 (Figure 2D–F) with or without live bacteria derived from Hudson River sediment. The data are expressed as molar percent from total content. Table 1 shows the PCB congeners corresponding to each peak and includes only congeners found in Aroclors above the detection limits. The methods used did not allow quantification of coplanar congeners independently of co-eluting congeners. Incubation with autoclaved (dead) bacteria yielded congener profiles of Auto1242 (Figure 2A) and Auto1254 (Figure 2D) that were identical to those of the respective Aroclors not incubated with autoclaved bacteria (not shown).

Microbial dechlorination of Aroclor 1242 occurred primarily from the *meta* position, with modest dechlorination from the *para* position and no evidence for the removal of *ortho*-substituted chlorines (Figure 2B). Hudson River microorganisms removed 35% of the chlorines of Aroclor 1242, corresponding to an average removal of 0.63 chlorines of the average 3.1 chlorines/biphenyl. Chlorobiphenyl (CB) congeners that appeared or increased more than 5 mol % of the total after dechlorination were those associated with peaks 1 (2-CB), 4 (2,2´-CB/2,6-CB), 7 (2,4-CB/2,5-CB), and 11 (2,2´,4-CB; Figure 2C). These peaks totaled 53.6 mol % after dechlorination (Figure 2B) but only 18.2 mol % before dechlorination (Figure 2A). The only peak that decreased by more than 5 mol % was peak 10 (2,2´,5-CB/4,4´-CB) (Figure 2C). It decreased from 8.6 mol % before dechlorination (Figure. 2A) to 3.4 mol % after dechlorination (Figure 2B).

Hudson River microorganisms removed 40% of the chlorines from Aroclor 1254, with an average removal of 1.2 chlorines of the average 4.96 chlorines/biphenyl in Aroclor 1254 (Figure 2E). The chlorines were removed primarily from the *meta* position, with modest dechlorination from the para position and no evidence for the removal of *ortho*-substituted chlorines. Congeners that appeared or increased more than 5 mol % of the total after dechlorination were those associated with peaks 4 (2,2´-CB/2,6-CB), 8 (2,2´,6-CB), 11 (2,2´,4-CB), 13 (2,2´,3-CB/ 2,4´,6-CB), and 26 (2,2´,4,4´-CB; Figure 2F). These peaks totaled 49.3 mol % after dechlorination (Figure 2E) but less than 2.5 mol % before dechlorination (Figure 2D). Congeners that decreased by more than 5 mol % of the total were those associated with peaks 38 (2,2´,3,5´,6-CB/ 2,3´,4,4-CB/ 2,2´,4,5,6´-CB), 42 (2,2´,4,5,5´-CB/ 2,2´,3,4´,5-CB), 49 (3,3´,4,4´-CB/ 2,3,3´,4´,6-CB), and 61 (2,2´,3,4,4´,5-CB/ 2,3,3´,4,5,6-CB; Figure 2F). They decreased from 36.1 mol % before dechlorination (Figure 2D) to 10.5 mol % after dechlorination (Figure 2E).

Therefore, bacterial incubations with Aroclor 1242 or Aroclor 1254 yielded PCB mixtures in which the major constituents were more lightly chlorinated, *ortho*-substituted, non-coplanar PCB congeners as compared with the nonmetabolized and parent commercial mixtures.

Stimulation of uterine contraction by bacterial metabolites of PCB mixtures. Uterine strips exposed to 100 µM Auto1242 (non-metabolized Aroclor 1242 mixture recovered from autoclaved bacterial cultures) exhibited significantly increased uterine contraction frequency compared with pretreatment (0 µM Auto1242) and

Table 1. Congener identities of chromatography peaks.

Peak no.	Congener(s)	Peak no.	Congener(s)	Peak no.	Congener(s)
1	2-CB	31	2,3´,4´,6-/2,2´,3,4-/2,3,4´,6-/2,3´,5,5´-CB	61	2,2´,3,4,4´,5´-/2,3,3´,4´,5,6-CB
2	3-CB	32	2,2´,3,6,6´-CB	62	2,3,3´,4,4´,6-CB
3	4-CB	33	2,2´,3,3´-CB	63	2,2´,3,3´,5,5´,6-CB
4	2,2´-/2,6-CB	34	2,3,3´,5-/2,3´,4,5-/2,2´,4,4´,6-/2,2´,4,5´,6-CB	64	2,2´,3,3´,4,5´,6-CB
5	2,4-/2,5-CB	35	2,3,3´,5-/2,3,4´,5-CB	65	2,2´,3,4´,5,5´,6-/2,2´,3,4,4´,5,6´-CB
6	2,3´-CB	36	2,4,4´,5-/2,2´,3,5,6´-CB	66	2,2´,3,4,4´,5´,6-CB
7	2,4´-/2,3-CB	37	2,3´,4´,5-/2´,3,4,5-CB	67	2,3´,4,4´,5,5´-CB
8	2,2´,6-CB	38	2,2´,3,5´,6-/2,2´,4,5,6´-/2,3´,4,4´-CB	68	2,2´,3,4,5,5´,6-CB
9	3,4-CB/3,4´-CB	39	2,3,3´,4-/2,2´,3,4´,6-CB	69	2,2´,3,3´,4,5,6´-/2,2´,3,4,4´,5,6-CB
10	2,2´,5-/4,4´-CB	40	2,2´,4,4´,6,6´-CB, surrogate	70	2,2´,3,3´,4´,5,6-CB
11	2,2´,4-CB	41	2,3,3´,4´-/2,3,4,4´-CB	71	2,2´,3,3´,4,4´,6-/2,3,3´,4,4´,5-/ 2,2´,3,3´,5,5´,6,6´-CB
12	2,3´,6-/2,3,6-CB	42	2,2´,4,5,5´-/2,2´,3,4´,5-CB		
13	2,2´,3-/2,4´,6-CB	43	2,2´,4,4´,5-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
14	2´,3,5-/2,2´,6,6´-CB	44	2,2´,3,4´,6,6´-/2,3,3´,5,6-/2,3´,4,4´,6-CB		
15	2,4,5-CB	45	2,2´,3´,4,5-/2,2´,3,4,5-/2,2´,3,5,6,6´-CB	73	2,2´,3,3´,4,5,5´-/2,3,3´,4,5,5´,6-CB
16	2,3´,5-CB	46	2,2´,3,4,5´-/2,3,4,4´,6-/2,3,3´,5,5´-CB	74	2,2´,3,4,4´,5,5´-CB
17	2,3´,4-CB	47	2,2´,3,4,4´-CB	75	2,3,3´,4´,5,5´,6-CB
18	2,4´,5-CB	48	2,2´,3,3´,6,6´,-CB	76	2,3,3´,4,4´,5´,6-CB
19	2,4,4´-/2,2´,4,6-CB	49	3,3´,4,4´-/2,3,3´,4´,6-CB	77	2,2´,3,3´,4,5,6,6´-CB
20	2´,3,4-/2,3,4-/2,3,3´-/2,2´,5,6´-CB	50	2,2´,3,5,5´,6-CB	78	2,2´,3,3´,4,4´,5-CB
21	2,4´,3-/2,2´,4,6´-CB	51	2,2´,3,3´,5,6´-/2´,3,4,5,5´-/2,2´,3,4,5´,6-CB	79	2,3,3´,4,4´,5,6-CB
22	2,2´,3,6-CB	52	2,3´,4,4´,5-/2,2´,3,4´,5´,6-/2,3,3´,4,5-CB	80	2,2´,3,3´,4´,5,5´,6-CB
23	2,2´,3,6´-CB	53	2,2´,3,3´,5,6-/2,2´,3,4,5,6´-/2,3,4,4´,5-CB	81	2,2´,3,3´,4,4´,5,6´-/2,2´,3,4,4´,5,5´,6-CB
24	2,2´,5,5´-/2,3´,5´,6-CB	54	2,2´,3,4´,5,5´-/2,3,3´,4,5´,6-CB	82	2,3,3´,4,4´,5,5´-CB
25	2,2´,4,5´-CB	55	2,2´,4,4´,5,5´-CB	83	2,2´,3,3´,4,4´,5,6-CB
26	2,2´,4,4´-CB	56	2,2´,3,3´,4,6´-/2,3,3´,4,4´-CB	84	2,2´,3,3´,4,5,5´,6,6´-CB
27	2,2´,4,5-/2,4,4´,6-CB	57	2,2´,3,4,5,5´-CB	85	2,2´,3,3´,4,4´,5,5´-CB
28	3,3´,4-CB	58	2,2´,3,3´,5,6,6´-CB	86	2,3,3´,4,4´,5,5´,6-CB
29	2,2´,3,5´-CB	59	2,2´,3,4,4´,5-CB	87	2,2´,3,3´,4,4´,5,5´,6-CB
30	2,2´,3,4´-/2,3,3´,6-/3,4,4´-CB	60	2,2´,3,3´,4,6,6´-CB		

CB, chlorobiphenyl.

lower concentrations of Auto1242 (3, 10, and 30 µM; Figure 3A; $p < 0.05$). The increased contraction frequency observed at 100 µM Auto1242 was 220.98 + 30.4%, similar to the response observed with 100 µM neat Aroclor 1242 (Aroclor 1242 that was not incubated with bacteria or sediment; Figure 1A). The responses to 10 µM and 30 µM Auto1242 were not significantly different from pretreatment (0 mM Auto1242). Exposure to HR1242 (PCBs recovered after incubation with Hudson River bacteria) shifted the concentration–response curve to the left. Uterine strips exposed to 10 mM or 30 mM HR1242 had contraction frequencies of 128.0 ± 16.0 and 339.4 ± 83.8%, respectively. These values were significantly increased compared with 10 µM or 30 µM Auto1242 and compared with pretreatment (0 µM HR1242; Figure 3A; $p < 0.05$). The response to 30 µM HR1242 was similar in magnitude to the response to 100 µM Auto1242 (Figure 3A) and 100 µM neat Aroclor 1242 (Figure 1A).

Comparable results were observed for Aroclor 1254. The Auto1254 nonmetabolized mixture, prepared by incubation of Aroclor 1254 with autoclaved microorganisms, did not stimulate contractions up to 300 µM (Figure 3B), consistent with neat Aroclor 1254 (Figure 1C). Due to limited availability of Auto1254, concentrations higher than 300 µM Auto1254 were not tested. The concentration–response curve of HR1254 was shifted to the left in comparison with Auto1254 (Figure 3B). Exposure to 30 µM or 50 µM HR1254 stimulated contractions to 153.8 ± 18.9 and 215.4 ± 22.5%, respectively, and these values were significantly increased compared with pretreatment (0 µM HR1254) and Auto1254 ($p < 0.05$). Because higher concentrations of HR1254 produced contractions with sustained increased basal tension, contraction frequency could not be quantified at concentrations above 50 µM HR1254.

Time-dependent stimulation of Aroclor 1242 on oscillatory contractions. Uterine strips were exposed to a single concentration of Aroclor 1242 for 1 hr to determine changes in the uterine contraction response with time. Aroclor 1242 increased contractions of uterine strips in a time-dependent as well as concentration-dependent manner (Figure 4A, right panel). Uterine strips treated with 50 µM or 100 µM Aroclor 1242 showed significant stimulation of contraction frequency compared with pretreatment (0 min exposure), starting at 30 min and continuing up to 60 min ($p < 0.05$). There were no significant differences between 50 µM and 100 µM Aroclor 1242-treated uterine strips. Aroclor 1242 had no effect on contractions at 10 µM with exposure durations up to 60 min. Although a modest stimulation was observed in earlier cumulative concentration–response experiments at 10 µM and 30 µM Aroclor 1242 in comparison with pretreatment (0 mM Aroclor 1242), these increases were not significant in comparison with solvent controls (Figure 1A), consistent with the response observed in this time-course experiment.

Sustained stimulation of contractions by Aroclor 1242 after rinsing. When uterine strips were exposed to 100 µM Aroclor 1242 for 1 hr followed by three rinses with PSS, the stimulatory effect was not readily reversible up to 4 hr after rinsing (Figure 4B). Also, a similar trend was observed with strips treated with 50 µM Aroclor 1242, but these differences were not statistically significant (Figure 4B).

Discussion

This study shows that the frequency of contraction of pregnant uteri was significantly increased in response to *in vitro* exposure to commercial and microbially dechlorinated PCB mixtures. Aroclor 1242 (42% chlorine content by weight) and Aroclor 1248 (48% chlorine content by weight) were more potent than Aroclor 1254 (54% chlorine content by weight). Similarly, the microbially dechlorinated Aroclor 1242 and 1254 mixtures, HR1242 and HR1254, were more potent than their parent commercial Aroclor mixtures. PCB mixtures with lower chlorine content and less chlorination of congeners had increased uterotonic activity. This is consistent with the findings of our previous study with individual congeners, in which 2,2´,4,4´,5,5´-CB exhibited no acute activity, and the more lightly chlorinated 2,4,6-CB elicited stronger stimulation of uterine contraction frequency compared with 2,2,4´,4´-CB (*28*). The relative uterotonic activity of Aroclor 1248 (48% chlorine content by weight) in comparison with Aroclor 1242 is not as clear. Although Aroclor 1248 was clearly more potent than Aroclor 1254, similar increases of contraction frequency were observed at 100 µM Aroclor 1242 and Aroclor 1248. However, greater uterotonic

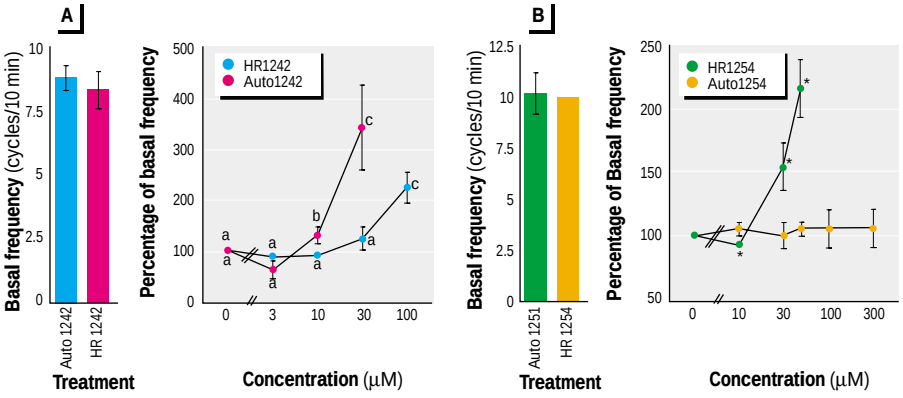

Figure 3. Concentration-dependent effects on uterine contraction frequency of (*A*) Aroclor 1242 or (*B*) Aroclor 1254 mixtures that were non-dechlorinated (Auto1242 and Auto1254, respectively) or microbially dechlorinated (HR1242 and HR1254, respectively). Left, basal contraction frequency of uterine strips before treatment with PCBs. Right, contraction frequency response to PCBs. Values shown are mean ± SEM. Means sharing the same letters are not significantly different ($p \leq 0.05$) for uterine strips exposed to Auto1242 or HR1242 ($n = 6$).
*Significantly different ($p \leq 0.05$) at the same concentration for uterine strips exposed to Auto1254 ($n = 2$) or HR1254 ($n = 5$).

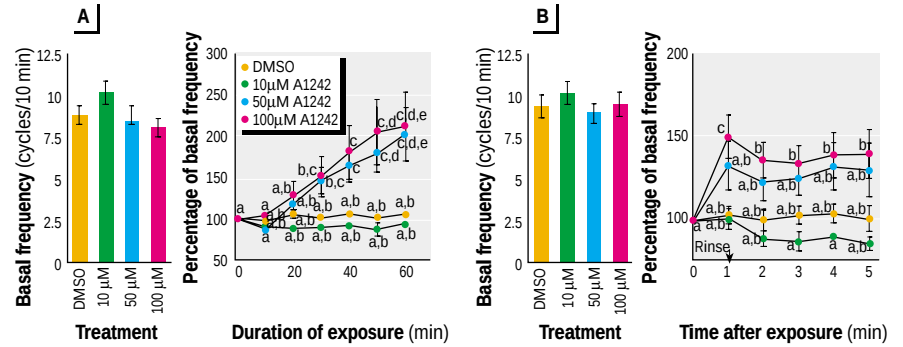

Figure 4. Time-dependent (*A*) stimulation of the frequency of uterine contraction by Aroclor 1242 (A1242) and (*B*) reversibility of Aroclor 1242 (A1242)-induced stimulation of uterine contraction frequency. Left, basal frequency of uterine strips before treatment with DMSO (solvent control) or Aroclor 1242. Right, contraction frequency response to Aroclor 1242. Values shown are mean ± SEM (n = 10–11). Means sharing the same letter are not significantly different ($p \leq 0.05$).

activity of Aroclor 1242 is suggested because the sustained contractions observed at 300 µM Aroclor 1242 were not observed at the same concentration of Aroclor 1248.

The reductive dechlorination of PCBs by bacteria found in sediments is an important factor in the environmental fate of PCBs (*31–33,37*) that is generally considered a part of the detoxification process. In the present study, incubation of Aroclor 1242 or Aroclor 1254 with bacteria eluted from Hudson River sediments produced dechlorinated product mixtures representative of those commonly found in anaerobic sediments (*31–33*). The major constituents of these microbially dechlorinated product mixtures were more lightly chlorinated, *ortho*-substituted, non-coplanar PCB congeners, as compared with the parent commercial mixtures. Dechlorination did not occur in the autoclaved bacteria controls, consistent with previous reports (*34*). The augmented stimulation of contraction of pregnant uterus by microbially dechlorinated PCB products observed in the present study suggests that microbial PCB dechlorination in PCB-contaminated sediment may generate PCBs with increased stimulatory activity towards uterine muscle. Whether this could result in meaningful exposures or risks to pregnant women is not known.

The total molar percentages of the main dechlorination products (2-CB, 2,2´-CB, 2,6-CB, 2,4´-CB, 2,4-CB, 2,2´,4-CB, 2,4,4´-CB, and 2,2´,4,4´-CB) follow the same general order as uterotonic activity (from greatest to least): HR1242, HR1254 > Aroclors 1242, 1248 > Aroclor 1254. It appears that the uterotonic activities of the PCB mixtures may depend on the relative abundance of lesser chlorinated *ortho*- and *para*-substituted congeners. This is consistent with the findings of our previous study with individual congeners, in which the more lightly chlorinated 2,4,6-CB and 2,2,4´,4´-CB stimulated uterine contraction frequency, whereas 3,3´,4,4´-CB, 3,3´,4,4´,5-CB, and 2,2´,4,4´,5,5´-CB did not (*28*).

Many studies have investigated relationships between the health effects of PCBs and the number and positions of chlorine substitutes on the biphenyl rings. Coplanar PCBs elicit a similar spectrum of biochemical and toxic responses as observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), related to their interaction with the aryl hydrocarbon (Ah) receptor (*2*). *Ortho*-substituted, non-coplanar PCBs, which were predominant in the most uterotonic PCB mixtures examined in the present study, do not have affinity to the Ah receptor but nonetheless evoke responses in various cells and tissues. For example, in rat cerebellar granule cells, *ortho*-substituted PCB congeners such as 2,2´-CB, 2,2´,4,6,6´-CB, 2,2´,5,5´-CB, and 2,2´,4,6-CB affect protein kinase C translocation, whereas coplanar congeners such as 3,3´,4,4´-CB and 3,3´,4,4´,5-CB do not (*38*). In addition, 2,2´-CB and 2,2´,4,6,6´-CB significantly increase intracellular calcium concentration of cerebellar granule cells whereas 4,4´-CB and 3,3´,4,4´,5-CB have a marginal effect or no effect (*39,40*). Accordingly, 2,2´,3,5´,6-CB induces Ca^{2+} release from sarcoplasmic reticulum isolated from rabbit muscle cells, but 3,3´,4,4´,5-CB does not alter Ca^{2+} transport (*41*). Furthermore, 2,2´,4,4´-CB but not 3,3´,4,4´-CB, stimulates degranulation of neutrophils, superoxide production, and inositol phosphates accumulation (*42,43*). In the uterus, the *ortho*-substituted congeners 2,4,6-CB and 2,2´,4,4´-CB stimulate frequency of contraction acutely, whereas the coplanar congeners 3,3´,4,4´-CB and 3,3´,4,4´,5´-CB do not (*28*). Thus, these reports show, for some responses, that *ortho*-substituted PCBs possess higher activity than coplanar congeners.

We previously characterized dechlorinated (by Hudson River microorganisms) and non-dechlorinated Aroclors as to their TCDD toxicity equivalencies (TEQ, an estimate of total Ah receptor binding) and found the order, from greatest to least, to be Aroclor 1254 > HR1254 > Aroclor 1248 > Aroclor 1242 > HR1242 (*44*). Except for the placement of HR1254, this is the reverse of the order of uterotonic activity found in the present study. This suggests that the uterotonic activity of the PCB mixtures is independent of Ah receptor binding. In this respect, our results for the PCB mixtures are consistent with our previously reported experiments with single PCB congeners in which the non-coplanar congeners 2,2´,4,4´-CB and 2,4,6-CB increased uterine contraction frequency and the Ah receptor agonists 3,3´,4,4´-CB and 3,3´,4,4´,5-CB did not (*28*).The inclusion of Aroclor 1016 or Aroclor 1221 in the present study may have provided clearer definition of the relative contribution of non-coplanar and coplanar congeners to the uterine response because these Aroclors contain little, if any, coplanar congeners (*30*).

When uterine strips were exposed to only a single concentration of Aroclor 1242 in the time-course experiment of the present study, 50 µM Aroclor 1242 was as uterotonic as 100 µM Aroclor 1242, suggesting that the response may be saturable. Moreover, the time-course data show that the frequency of contraction increased with duration of exposure to Aroclor 1242 up to 60 min. These data indicate that either the PCBs required this time to penetrate the tissue sufficiently to elicit a maximal effect or that the response required this time for maximal activation. In addition, these results indicate that the uterine response at any given concentration in the cumulative concentration–response experiments is a function of time as well as concentration because PCBs were added to the muscle baths at 20-min intervals. As a consequence, the data from the cumulative concentration–response experiments may not reveal the maximal response at any particular concentration. Nonetheless, the cumulative concentration-response design was useful for determining initial effective concentration ranges and for comparing responses to the limited quantities of PCB mixtures available from the bacterial cultures.

We used DMSO as the solvent for the PCB mixtures. In the cumulative concentration–response experiments, DMSO was added to the muscle baths of control uterine strips in a cumulative manner at identical concentrations used to deliver the PCB mixtures. Although the increases in uterine contraction frequency were statistically significant compared with pretreatment (0 µM DMSO) in the DMSO-exposed solvent controls for the Aroclor 1242 experiment shown in Figure 1A, these increases were modest, and this was the only set of controls to show this pattern. Because DMSO stimulation of contraction frequency has not been reported previously and was not replicated in other solvent control groups in the present study, we suggest that the increased frequency of contraction observed in the DMSO-exposed controls shown in Figure 1A most likely reflects variability of the uterine strips and experimental conditions, rather than uterotonic activity of DMSO.

In the same experiment in which increased contraction frequency was observed in response to increased concentrations of DMSO, the frequency of contraction also increased, in the Aroclor 1242-treated strips at 10 µM and 30 µM Aroclor 1242 relative to 0 µM Aroclor 1242 (Figure 1A). In contrast, significant increases of contraction frequency were not observed with 10 mM Aroclor in the time-course experiment (Figure 4A), nor were significant increases relative to pretreatment (0 µM Auto1242) observed in strips exposed to 10 µM or 30 µM Auto1242 (Figure 3A), even though the PCB congener HPLC profile of Auto1242 was identical to that of Aroclor1242. One possible explanation for this discordance of results is that the lower basal contraction frequency in the Aroclor 1242-exposed group (Figure 1A) may have allowed statistical detection of smaller absolute changes in contraction frequency. Alternatively, because the cumulative concentration–response experimental design is confounded by time, the observed increase of frequency may be a reflection of improved contractility as the tissue accommodated to the muscle bath

environment. This possibility is suggested by the parallel increased contraction frequencies in the Aroclor-treated and DMSO (solvent) controls. Furthermore, the increases of contraction frequency at 10 and 30 μM Aroclor 1242 were relatively slight and were not statistically different from solvent controls, even though they were statistically significant from 0 μM Aroclor 1242. Based on these considerations, we suggest that the response to 100 μM Aroclor 1242 is the only meaningful Aroclor 1242-induced stimulation in the experiment shown in Figure 1A.

Several previous studies suggest that PCBs may interfere with the ability to maintain pregnancy to term. However, a cause-and-effect relationship between PCBs and preterm birth is by no means certain. Increased blood or blood serum PCB levels were found in women with no identified exposure to PCBs who delivered prematurely compared to women who went to term (*3*–*5*). In addition, small but statistically significant decreases in gestation length were observed in women occupationally exposed to PCBs (*6,7*) or exposed to PCBs by consumption of contaminated fish from Lake Michigan (*8*) or the Baltic Sea (*9*). An increased incidence of premature birth was also observed in the Yu-Cheng Taiwanese babies born to mothers exposed to PCBs, as well as to polychlorinated dibenzofurans and polychlorinated quarterphenyls, through consumption of contaminated rice oil (*10*). However, a study of New York women with no identified source of PCB exposure found no significant differences in blood serum PCB concentrations of women who delivered prematurely compared to women who did not (*11*), and other studies failed to detect a relationship between premature birth and consumption of PCB-contaminated Great Lakes fish (*12,13*). One study reported a positive association (*4*), whereas another study found no evidence of a significant association (*14*) between PCB exposure and spontaneous abortion (extremely premature delivery of a nonviable fetus) in women. Because birth weight is correlated with gestational age at birth and is easier to record accurately than gestational age, it is sometimes used as a surrogate measure of gestation length, but it can be confounded by intrauterine growth retardation. Decreased birth weight has been associated with PCB exposure in some epidemiologic studies (*9,45*–*47*) but not in others (*12,48,49*).

In the United States, the incidence of preterm births (infants born before 37 completed weeks of gestation) has risen 17% since 1981 to the current rate of 11% of live births (*57*). Preterm births account for 85% of early infant deaths and annual costs of $5 billion were estimated in 1994 for intensive neonatal care of premature infants (*58*). Despite its potential significance, the contribution of environmental exposures to prematurity is poorly understood. The present study describes PCB-induced stimulation of contraction frequency in isolated midgestation uterus, a response that would be expected to promote initiation of parturition if it occurred *in vivo*. Although the midgestation uterus may be an appropriate model for spontaneous abortion, it may not be relevant for uterine responses later in gestation such as preterm birth because the uterus undergoes significant modifications as pregnancy advances. Nonetheless, preliminary experiments in our laboratory indicate that late-gestation rat uterus (day 20) responds to PCBs in a manner similar to midgestation uterus. Additional experiments with late-gestation uterus should provide greater relevance to preterm birth.

In the study by Taylor et al. (*7*) of occupationally exposed women with decreased gestational length, the geometric mean of serum PCB concentrations was 302 ppb. In women whose decreased gestational length was associated with consumption of contaminated Lake Michigan fish, the total PCBs in serum averaged 5.5 ng/mL. A more recent study found that the total chlorobiphenyls analyzed in blood plasma of Swedish women ranged from 1.0 to 10.7 ng/g (fresh weight) (*50*), and a recent study of five American pregnant women found PCB concentrations in maternal blood ranging from 18 to 50 pg/g (lipid based) (*51*). Differences in reported concentrations are likely related to different PCB exposures of the study populations and to differences in the analytical procedures used, especially if the PCB concentrations are reported on a lipid weight basis (*50*). Regardless, the micromolar concentrations that were required in the present study to elicit uterine responses are much greater than the concentrations reported in human samples. However, direct comparisons of concentrations used in the present experiment with concentrations observed in human tissue samples are difficult due to inherent substantive differences between the *in vitro* and *in vivo* conditions. For example, the physiologic salt solution that bathed the uterine strips in the muscle baths in the present study was an aqueous solution lacking proteins and lipids. This contrasts with blood and other tissues, in which the presence of lipids and proteins likely plays an important role in determining PCB concentrations and biological activity. Moreover, humans are exposed to low concentrations of PCBs over extended periods of time, allowing for tissue accumulation, whereas the muscle bath experiments in the present study were performed under acute exposure conditions. PCBs do concentrate in the uterine muscle *in vivo*, particularly during pregnancy (*26,27*), further complicating comparisons. In addition, because the metabolic similarities or dissimilarities of the pregnant rat uterus and human uterus are largely unexplored, it is not known whether species differences in uterine metabolism may alter the response. Furthermore, the lack of circulation and extrauterine metabolism *in vitro* may influence PCB distribution and uterine responses in unknown ways. For these reasons, it is difficult to extrapolate from the concentrations used in the present study to environmental exposure levels.

Various effects on parturition have been reported in laboratory animal studies of PCB exposure during pregnancy. Monkeys exposed during pregnancy to the PCB mixture Aroclor 1254 or Aroclor 1248 at doses toxic to the mother (5 ppm in diet) aborted their pregnancies (*19,20*). After discontinuation of PCB exposure, a subsequent breeding experiment showed that the monkeys exposed to 5 ppm Aroclor 1248 gave birth to smaller infants, even though the general health of the females had improved substantially (*52*). In rats, the PCB congeners 3,3′,4,4′-CB (*16,17*) and 2,2′-CB (*18*), as well as the mixture Aroclor 1254 (*53*), increased gestation length. In guinea pigs exposed to the PCB mixture Clophen 50, increases in the variation of gestation length and incidence of difficult labor were observed (*15*). In the wild, significantly higher tissue concentrations of PCBs were reported in California sea lions that delivered prematurely compared with sea lions that delivered full-term pups (*21*). Uterine motility was not assessed in any of these animal studies. In future experiments, it would be interesting to examine uterine contractility in PCB-exposed animals to determine if the *in vitro* responses observed in the present study occur *in vivo*.

With the discontinuation of production of PCBs, the risk of exposure to commercial mixtures of PCBs is minimal. More likely, humans are apt to be exposed to those PCB congeners that persist in the environment and bioaccumulate in the food chain. Consequently, the Aroclor mixtures used in this study do not represent PCB mixtures likely to be encountered through environmental contamination. The augmented stimulation of contraction of pregnant uterus by microbially dechlorinated PCB products observed in the present study suggests that microbial PCB dechlorination in PCB-contaminated sediment may generate PCBs with increased stimulatory activity toward uterine muscle. However, the concentrations required to stimulate uterine contraction frequency are higher than concentrations likely to be encountered in the environment.

This study shows, for the first time, that PCB mixtures are uterotonic and that

increased uterotonic activity is associated with decreased chlorine content and increased abundance of lesser chlorinated *ortho*- and *para*-substituted congeners in PCB mixtures. Moreover, the results demonstrate that microbial dechlorination markedly increases the uterotonic activity of commercial PCB mixtures. Because of the relatively high concentrations required to stimulate uterine contraction frequency *in vitro*, the complexity of comparing PCB mixtures, and the differences between the *in vitro* exposure conditions of the present study and exposure conditions for human populations, it is inappropriate to draw direct conclusions to human health. Nonetheless, it is interesting that several previous studies found a significant association between PCB exposure and decreased gestation length in women (*3–10*) and animals (*19–21*). If PCBs stimulate contraction frequency in the late gestation uterus *in vivo*, this may be a mechanism by which PCBs could decrease gestation length.

References and Notes

1. Muir DC, Norstrom RJ. Geographical differences and time trends of persistent organic pollutants in the Arctic. Toxicol Lett 112–113:93–101 (2000).
2. Safe SH. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. CRC Crit Rev Toxicol 24:87–149 (1994).
3. Bercovici B, Wassermann M, Cucos S, Ron M, Wassermann D, Pines A. Serum levels of polychlorinated biphenyls and some organochlorine insecticides in women with recent and former missed abortions. Environ Res 30:169–174 (1983).
4. Leoni V, Fabiani L, Marinelli G, Puccetti G, Tarsitani GF, De Carolis A, Vescia N, Morini A, Aleandri B, Pozzi V, et al. PCB and other organochlorine compounds in blood of women with or without miscarriage: a hypothesis of correlation. Ecotoxicol Environ Saf 17:1–17 (1989).
5. Wassermann M, Ron M, Bercovici B, Wassermann D, Cucos S, Pines A. Premature delivery and organochlorine compounds: polychlorinated biphenyls and some organochlorine insecticides. Environ Res 28:106–112 (1982).
6. Taylor PR, Lawrence CE, Hwang HL, Paulson AS. Polychlorinated biphenyl: influence on birth weight and gestation. Am J Public Health 74:1153–1154 (1984).
7. Taylor PR, Stelma JM, Lawrence CE. The relation of polychlorinated biphenyls to birth weight and gestational age in the offspring of occupationally exposed mothers. Am J Epidemiol 129:395–406 (1989).
8. Fein GG, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. J Pediatr 105:315–320 (1984).
9. Rylander L, Stromberg U, Hagmar L. Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birthweight. Scand J Work Environ Health 22:260–266 (1996).
10. Yen YY, Lan SJ, Yang CY, Wang HH, Chen CN, Hsieh CC. Follow-up study of intrauterine growth of transplacental Yu-Cheng babies in Taiwan. Bull Environ Contam Toxicol 53:633–641 (1994).
11. Berkowitz GS, Lapinski RH, Wolff MS. The role of DDE and polychlorinated biphenyl levels in preterm birth. Arch Environ Contam Toxicol 30:139–141 (1996).
12. Dar E, Kanarek MS, Anderson HA, Sonzogni WC. Fish consumption and reproductive outcomes in Green Bay, Wisconsin. Environ Res 59:189–201 (1992).
13. Lonky E, Reihmann J, Darvill T, Mather J, Daly H. Neonatal behavioral assessment scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. J Great Lakes Res 22:198–212 (1996).
14. Mendola P, Buck GM, Vena JE, Zielezny M, Sever LE. Consumption of PCB-contaminated sport fish and risk of spontaneous fetal death. Environ Health Perspect 103:498–502 (1995).
15. Lundkvist U. Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. Toxicology 61:249–257 (1990).
16. White RD, Allen SD, Bradshaw WS. Delay in the onset of parturition in the rat following prenatal administration of developmental toxicants. Toxicol Lett 18:185–192 (1983).
17. Rands PL, White RD, Carter MW, Allen SD, Bradshaw WS. Indicators of developmental toxicity following prenatal administration of hormonally active compounds in the rat. I. Gestational length. Teratology 25:37–43 (1982).
18. Torok P. Delayed pregnancy in NMRI mice treated with PCB:2,2′-dichlorobiphenyl. Bull Environ Contam Toxicol 16:33–36 (1976).
19. Arnold DL, Mes J, Bryce F, Karpinski K, Bickis MG, Zawidzka ZZ, Stapley R. A pilot study on the effects of Aroclor 1254 ingestion by Rhesus and Cynomolgus monkeys as a model for human ingestion of PCBs. Food Chem Toxicol 28:847–857 (1990).
20. Barsotti DA, Marlar RJ, Allen JR. Reproductive dysfunction in Rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Food Cosmet Toxicol 14:99–103 (1976).
21. DeLong R, Gilmartin WG, Simpson JG. Premature births in California sea lions: association with high organochlorine pollutant residue levels. Science 181:1168–1170 (1973).
22. Olson DM, Mijovic JE, Sadowsky DW. Control of human parturition. Semin Perinatol 19:52–63 (1995).
23. Bell R. The prediction of preterm labour by recording spontaneous uterine activity. Br J Obstet Gynecol 90:884–887 (1983).
24. Katz M, Newman RB, Gill PJ. Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery. Am J Obstet Gynecol 154:44–47 (1986).
25. Main DM, Katz M, Chiu G, Campion S, Gabbe SG. Intermittent weekly contraction monitoring to predict preterm labor in low-risk women: a blinded study. Obstet Gynecol 72:757–761 (1988).
26. Polishuk ZW, Wassermann D, Wassermann M, Cucos S, Ron M. Organochlorine compounds in mother and fetus during labor. Environ Res 13:278–284 (1977).
27. Lindenau A, Fischer B, Seiler P, Beier HM. Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. Human Reprod 9:772–780 (1994).
28. Tsai ML, Webb RC, Loch-Caruso R. Congener-specific effects of PCBs on contractions of pregnant rat uteri. Reprod Toxicol 10:21–28 (1996).
29. Frame GM, Wagner RE, Carnahan JC, Brown JF Jr, May RJ, Smullen LA, Bedard DL. Comprehensive, quantitative, congener-specific analyses of eight Aroclors and complete PCB congener assignments on DB-1 capillary GC columns. Chemosphere 33:603–623 (1996).
30. ATSDR. Toxicological Profile for Polychlorinated Biphenyls (Update). Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1998.
31. Bedard DL, Wagner RE, Brennan MJ, Haberl ML, Brown JFJ. Extensive degradation of Aroclors and environmentally transformed polychlorinated biphenyls by *Alcaligenes eutrophus* H850. Appl Environ Microbiol 53:1094–1102 (1987).
32. Tiedje JM, Quensen JF III, Chee-Sanford J, Schimel JP, Boyd SA. Microbial reductive dechlorination of PCBs. Biodegradation 4:231–240 (1993–1994).
33. Unterman R, Bedard DL, Brennan MJ, Bopp LH, Mondello FJ, Brooks RE, Mobley DP, McDermott JB, Schwartz CC, Dietrich DL. Biological approaches for polychlorinated biphenyl degradation. Basic Life Sci 45:253–269 (1988).
34. Mousa MA, Quensen JF III, Chou K, Boyd SA. Microbial dechlorination alleviates inhibitory effects of PCBs on mouse gamete fertilization *in vitro*. Environ Sci Tech 30:2087–2092 (1996).
35. Quensen JF III, Boyd SA, Tiedje JM. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl Environ Microbiol 56:2360–2369 (1990).
36. Juberg DR, Webb RC, Loch-Caruso R. Characterization of *o,p′*-DDT-stimulated contraction frequency in rat uterus in vitro. Fundam Appl Toxicol 17:543–549 (1991).
37. Yadav JS, Quensen JF III, Tiedje JM, Reddy CA. Degradation of polychlorinated biphenyl mixtures (Aroclors 1242,1254, and 1260) by the white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis. Appl Environ Micorbiol 61:2560–2565 (1995).
38. Kodavanti PR, Ward TR, McKinney JD, Tilson HA. Increased [^{3}H]phorbol ester binding in rat cerebellar granule cells by polychlorinated biphenyl mixtures and congeners: structure-activity relationships. Toxicol Appl Pharmacol 130:140–148 (1995).
39. Kodavanti PR, Shin DS, Tilson HA, Harry GJ. Comparative effects of two polychlorinated biphenyl congeners on calcium homeostasis in rat cerebellar granule cell. Toxicol Appl Pharmacol 123:97–106 (1993).
40. Mundy WR, Shafer TJ, Tilson HA, Kodavanti PR. Extracellular calcium is required for the polychlorinated biphenyl-induced increase of intracellular free calcium levels in cerebellar granule cell culture. Toxicology 136:27–39 (1999).
41. Wong P, Pessah I. *Ortho*-substituted polychlorinated biphenyls alter calcium regulation by a ryanodine receptor-mediated mechanism: structural specificity toward skeletal- and cardiac-type microsomal calcium release channels. Mol Pharmacol 49:740–751 (1996).
42. Brown AP, Ganey PE. Neutrophil degranulation and superoxide production induced by polychlorinated biphenyls are calcium dependent. Toxicol Appl Pharmacol 131:198–205 (1995).
43. Tithof PK, Schiamberg E, Peters-Golden M, Ganey PE. Phospholipase A_2 is involved in the mechanism of activation of neutrophils by polychlorinated biphenyls. Environ Health Perspect 104:52–58 (1996).
44. Quensen JF III, Tiedje JM, Boyd SA, Enke C, Lopshire R, Giesy J, Mora M, Crawford R, Tillet D. Evaluation of the suitability of reductive dechlorination for the bioremediation of PCB-contaminated soils and sediments. In: International Symposium, Soil Decontamination Using Biological Processes, 6–9 December 1992, Karlsruhe, Germany. Frankfurt am Main, Germany:Dechema, 1992;91–100.
45. Rylander L, Stromberg U, Hagmar L. Decreased birthweight among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 21:368–375 (1995).
46. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A. Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. HSMHA Health Rep 86:1083–1091 (1971).
47. Patandin S, Koopman-Esseboom C, de Ridder MA, Weisglas-Kuperus N, Sauer PJ. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 44:538–545 (1998).
48. Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tinglestad J, Tully M. Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr 109:335–341 (1986).
49. Vartiainen T, Jaakkola JJK, Saarikoski S, Tuomisto J. Birth weight and sex of children and the correlation to the body burden of PCDDs/PCDFs and PCBs of the mother. Environ Health Perspect 106:61–66 (1998).
50. Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Stromberg U, Hagmar L, Ostman C. Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption. Arch Environ Contam Toxicol 32:329–336 (1997).
51. Schecter A, Kassis I, Papke O. Partitioning of dioxins, dibenzofurans, and coplanar PCBs in blood, milk, adipose tissue, placenta and cord blood from five American women. Chemosphere 37:1817–1823 (1998).
52. Allen JR, Barsotti DA, Carstens LA. Residual effects of polychlorinated biphenyls on adult nonhuman primates and their offspring. J Toxicol Environ Health 6:55–66 (1980).
53. Brezner E, Terkel J, Perry AS. The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat—I. Comp Biochem Physiol C 77:65–70 (1984).