PCB77 dechlorination mixtures alter proinflammatory events in vascular endothelial cells

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Abstract

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are a high priority for remediation at Superfund sites throughout the United States. In a real world context, complete dechlorination is difficult to achieve. Using vascular endothelial cells as a sensitive model for PCB-induced vascular toxicity, we hypothesized that PCB dechlorination to biphenyl would reduce biological activity and toxicity. Thus, in addition to PCB 77, we assessed the toxicity of intermediate dechlorination mixtures. Specific dechlorination mixtures representing remediation products at 0 (PCB 77), 10, 24, and 48 (biphenyl) hours were prepared. Treatment groups resulting from 24 and 48 hour dechlorination showed decreased superoxide production and nuclear factor kappa B (NFκB) activation in our cell model, while endothelial cell viability improved. All dechlorination mixtures which contained the parent PCB, and independent of its concentration, contributed to a significant increase in cytochrome P450 1A1 (CYP1A1) mRNA and protein expression. The presence of PCB 77 in any treatment group was significantly linked to increased expression of vascular cell adhesion molecule 1 (VCAM1) and monocyte chemoattractant protein-1 (MCP-1). To test whether PCB 77 alone was responsible for the inflammatory response, we also prepared dechlorination mixtures lacking PCB 77. Our data suggest that in addition to pure PCB 77, dechlorination products (resulting from partial dechlorination at 10 hours) can induce some inflammation in vascular endothelial cells. These data suggest that dechlorination of PCBs is beneficial but that remediation products should be tested to determine which degree of remediation sufficiently attenuates biological toxicity.

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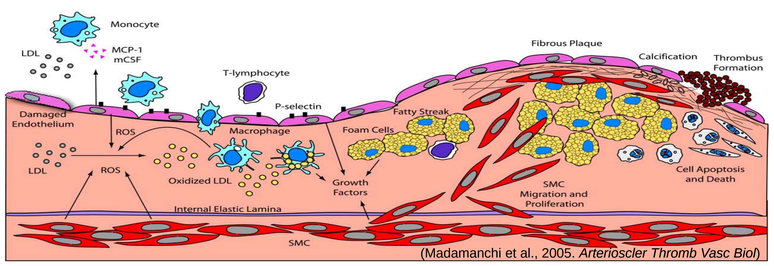
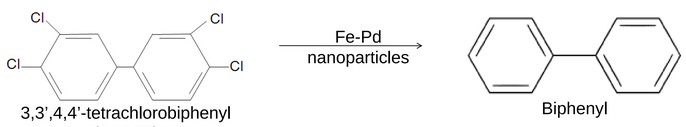
Background

- In the vascular endothelium, coplanar PCBs, such as PCB 77, activate the aryl hydrocarbon receptor (AHR) generating reactive oxygen species (ROS) and stimulating pro-inflammatory endpoints including cytochrome P450 1A1 (CYP1A1), which is linked to higher levels of cellular ROS, and of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule (VCAM1), which both contribute to inflammation (Majkova *et al.*, 2010; Venkatachalam *et al.*, 2008).



(Madamanchi et al., 2005. *Arterioscler Thromb Vasc Biol*)

- Previously, it was demonstrated that biphenyl, the major end product of PCB77 dechlorination, was non-toxic in primary endothelial cells (Venkatachalam *et al.*, 2008).



3,3',4,4'-tetrachlorobiphenyl (PCB77)

Biphenyl

Methods

- ***Cell culture and treatment:*** Cell culture assays used primary endothelial cells that were isolated from porcine pulmonary arteries as previously described (Toborek *et al.*, 2002). Cells were maintained in M199, containing 10% fetal bovine serum (FBS) and 2 mM L-glutamine, 50 μg/mL streptomycin, 50 units/mL penicillin. The treatment media contained 1% FBS and the treatment of either vehicle (DMSO), PCB remediation mixture (Table 1), or individual PCB.
- ***MTT Assay:*** Treated cell cultures were tested for viability using the Vybrant MTT Cell Proliferation Assay Kit (Life Technologies, Grand Island, NY). Formazan was added using the DMSO method. A SpectraMax M2 Microplate Reader (Molecular Devices, Sunnyvale, CA) was used for absorbance readings.
- ***NFκB Activation:*** Nuclear extracts were isolated as previously described (Lim *et al.*, 2007). NFκB activation was performed using the DNA-protein binding detection kit (Gibco-BRL, Grand Island, NY) with radiolabeled oligonucleotides (Han, S.S. et al, 2010).
- ***Superoxide Assay:*** Primary endothelial cells were cultured on multi-chambered slides and treated as described above. The cells were then treated with DHE and fixed as described previously (Han et al., 2010). Fluorescence was detected by an Olympus BX61W1 fluorescence microscope and quantified using ImageJ 1.42q software (NIH, Bethesda, MD).
- ***Real time – PCR:*** mRNA was isolated from cell cultures using the Trizol® method. Isolated mRNA was resuspended in RN/DNAse free water and quantified using UV-vis spectroscopy at 260nM. cDNA was made using a reverse transcriptase reaction. CYP1A1, MCP-1, VCAM1, and β-actin ratios were analyzed with using SYBR® Green reagent.(Han et al., 2010 and Makojva et al., 2009).
- ***ELISA:*** Cell culture media was removed from treated cell cultures, and an ELISA was performed according to the manufacturer's directions for the Quantikine ELISA kit (R&D Systems, Minneapolis, MN).
- ***Statistical analysis:*** Comparisons between treatments were made by one-way analysis of variance followed by Tukey's post-hoc test using SigmaPlot 12.0 software. Statistical probability of P<.05 was considered significant.

Results

Treatment mixtures based on remediation data

Table 1. Treatments representing the remediation mixtures. Cells were treated with (1) vehicle (DMSO) , (2) PCB 77 at 0 hrs. of remediation, (3) remediation mixture after 10hrs. of dechlorination, (5) remediation mixture after 24hrs. of dechlorination, and (8) fully dechlorinated biphenyl after 48hrs. of remediation. Additional test mixtures included (4) mixture after 10 hrs. of dechlorination without PCB 77, (6) mixtures after 24 hrs. of dechlorination without PCB 77, (7) mixture after 24 hrs. of remediation with 10 h mixture PCB 77 concentration, (9) concentration of PCB 77 alone based on 10 hrs of dechlorination, and (10) concentration of PCB 77 alone based on 24 hrs of dechlorination.

	1	2	3	4	5	6	7	8	9	10
Mixture Components	DMSO (0.1 % v/v Vehicle)	0 h (μM)	10 h (μM)	10 h without PCB 77 (μM)	24 h (μM)	24 h without PCB 77 (μM)	24 h with addition of 10 h PCB 77 (μM)	48 h (μM)	10 h Concentration of PCB 77 (μM)	24 h Concentration of PCB 77 (μM)
PCB 77	—	5	0.7	—	0.2	—	0.7	—	0.7	0.2
PCB 37	—	—	0.2	0.2	0.1	0.1	0.1	—	—	—
PCB 35	—	—	0.2	0.2	0.1	0.1	0.1	—	—	—
PCB 15	—	—	0.5	0.5	0.4	0.4	0.4	—	—	—
PCB 13	—	—	0.3	0.3	0.3	0.3	0.3	—	—	—
PCB 12	—	—	0.3	0.3	0.3	0.3	0.3	—	—	—
PCB 11	—	—	0.5	0.5	0.4	0.4	0.4	—	—	—
PCB 03	—	—	0.1	0.1	0.1	0.1	0.1	—	—	—
PCB 02	—	—	0.1	0.1	0.1	0.1	0.1	—	—	—
Biphenyl	—	—	1.9	1.9	3.1	3.1	3.1	5	—	—
Total Concentration	—	5	5	4.3	5	4.8	5.5	5	0.7	0.2

Dechlorination mixtures stimulate the production of ROS in endothelial cells

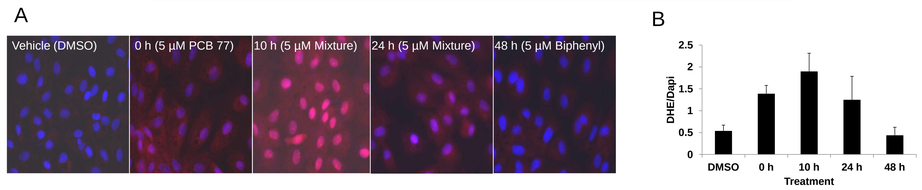


Figure 2. Dechlorination mixtures cause the production of Superoxide in endothelial cells. Cells were treated with dechlorination mixtures and DMSO and were incubated for 4hrs. Cells were then treated with N,N'-(1,2-dihydroxyethylene)bisacrylamide (DHE), fixed, fluorescent images were recorded (A) ,and fluorescence was quantified (B).

Dechlorination mixtures increase CYP1A1 and VCAM1 mRNA at 24hrs

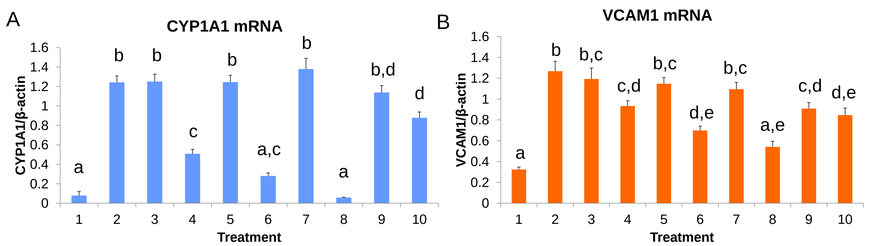


Figure 4. Treatments containing PCB 77 induce significant changes in mRNA expression; dechlorinated mixtures have an attenuated response. (A) CYP1A1 mRNA expression was significantly increase for treatments 2, 3, 4, 5, 7, 9, and 10. Treatment 4 (10 h without PCB 77) was significantly less than PCB 77 containing groups but increased over control. Treatments 6 and 8 were not different from control. (B) VCAM1 mRNA expression was significantly increased in PCB 77 containing groups. Treatments 4 and 6 were significantly increased over control, and treatment 8 (biphenyl) was not different from control. Significantly different for Tukey's all-pairwise analysis at $P < 0.05$.

Cell viability after exposure to dechlorination mixtures

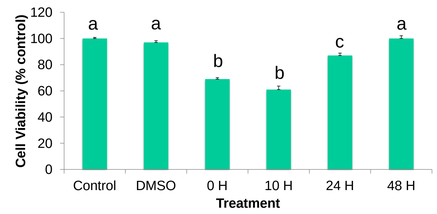


Figure 1. PCB 77 causes a decrease in cell viability that is attenuated by dechlorination. Cells were untreated (control) or treated with DMSO, 5 μM PCB77 (0 h), 10 h, 24 h, or 48 h and incubated for 24hrs. DMSO and biphenyl (48 h) did not have significant decreases in viability. 0 h and 10 h treatments had significant changes in viability. The 24 h treatment produced a significant decrease in viability, but this decrease was less than the 0 h and 10 h. Significantly different compared with Tukey's all-pairwise at $P < 0.05$.

NFκB binding after exposure to dechlorination mixtures

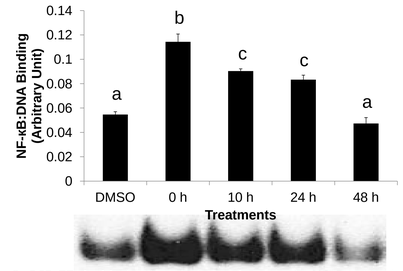


Figure 3. PCB 77 dechlorination causes a decrease in NFκB activation. Cells were treated with vehicle control (DMSO), 0 h (PCB 77, 5 μM) 10 h, 24 h and 48 h, and incubated for 4 h. The 0 h treatment exhibited the highest level of DNA binding. The 10 h and 24 h treatments were significantly greater than vehicle or biphenyl but had significantly less binding than that of 0 h. Results are the mean ± SEM. Significantly different at $P < 0.05$ and analyzed by one-way ANOVA with Tukey 's post hoc.

MCP-1 protein expression in media after 24 hr exposure

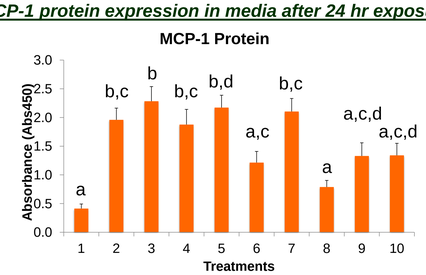


Figure 5. Dechlorination mixtures cause an increase in MCP-1 protein expression. Cells were treated with all the mixtures and incubated for 24hrs. Cell media was collected and protein levels were measured by ELISA. Treatments 2, 3, 4, 5, and 7 were significantly increased over DMSO control. Treatments 6, 9, and 10 exhibited an intermediate response between DMSO and the other treatments. Biphenyl was not different from DMSO. Significant difference compared based on Tukey's all-pairwise analysis at $P < 0.05$.

Conclusions

- The parent compound, PCB 77, even at low concentrations, significantly upregulates inflammatory markers.
- Evidence suggests that the intermediate remediation products may contribute to toxicity within the cell.
- For this reason, testing of remediation mixtures will continue to be an important aspect of environmental remediation.

NIEHS National Institute of Environmental Health Sciences U.S. National Institutes of Health

Srp Superfund Research Program

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