Altered Biological activity of Commercial PCB Mixtures Due to Microbial Reductive Dechlorination

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ABSTRACT

This study is part of a multidisciplinary effort to assess changes in the spectrum of toxicity of contaminants resulting from biological or chemical remediation processes. Our initial focus has been directed toward commercial PCB mixtures (Aroclors) that have undergone microbial reductive dechlorination. This process involves the removal of chlorine directly from biphenyl moiety and replacement with hydrogen, and results in a dechlorinated product mixture in which the average number of chlorines per biphenyl is reduced. Generally chlorine is most readily removed from the *meta* and *para* positions. Aroclors were dechlorinated by anaerobic microorganisms eluted from different PCB-contaminated sediments. The biological activities of the dechlorinated products were assessed by measuring their effects on mouse gamete fertilization, Ah receptor activity, induction of AP-1 transcription factor activity, insulin release from RINm5F cells, and neutrophil activation. Microbial dechlorination of Aroclors 1242 and 1254 reduced or eliminated the inhibitory effect of these mixtures on *in vitro* fertilization of mouse gametes. A significant decrease in the Ah receptor mediated activity, measured by EROD induction, was also observed for the dechlorinated Aroclors. This was likely due to a significant reduction (10 to 100 fold) in the concentration of coplanar PCB congeners in the dechlorinated product mixtures. PCBs were shown previously to activate PKC isozymes causing an induction of AP-1 activity. The microbially dechlorinated Aroclors showed a decreased ability to activate AP-1 transcription factor. Each of these studies indicated that the toxic effects of PCB mixtures may be mitigated by microbial reductive dechlorination. In contrast, dechlorinated Aroclor 1254 had similar or slightly greater potential than unaltered 1254 for producing insulin release from cell line RINm5F. Also, neutrophil activation by PCBs was not found to be altered when the products of anaerobic dechlorination were evaluated. Together these data indicate that PCB biological activity is altered by microbial dechlorination, and alteration is dependent on the biological system used to assess activity.

OBJECTIVES

The objective of this study was to evaluate changes in the biological activities of commercial PCB mixtures after the microbial dechlorination of these mixtures. Microbial reductive dechlorination involves removal of chlorine directly from biphenyl and replacement with hydrogen and results in a dechlorinated product mixture in which the average number of chlorines per biphenyl

is reduced. PCB dechlorination has been observed *in situ* at several sites including the Hudson River (NY), Silver Lake (MA), Sheboygan River (WI), Waukegan Harbor (IL), Acushnet Estuary (MA), Hoosic River (MA) and River Raisin (MI). Microbial transformation of PCBs has potential utility for bioremediation of PCBs. For biological remediation to be effective, the end products must have reduced biological activities compared to the parent material. Accordingly, the assessment of the changes in the spectrum of toxicity resulting from biological remediation of environmental contaminants is critical before considering the application of bioremediation. In addition, to accurately asses at sites where PCBs have undergone reductive dechlorination, the toxicity of the resultant product mixture must be known.

In this study, we have conducted large-scale, long-term biological incubations under anaerobic conditions to produce sufficient quantities of dechlorinated PCBs with known composition. We then evaluated the biological activities of the resulting PCB mixtures before and after dechlorination using a battery of toxicological end points including *in vitro* fertilization, EROD induction, induction of AP-1 transcription activity, insulin release and neutrophil activation.

METHODS AND RESULTS

A. Microbial dechlorination of PCBs

Microbially dechlorinated products from Aroclors 1242 and 1254 were generated in long term anaerobic incubations, extracted, purified and quantified. Microorganisms eluted from PCB-contaminated River Raisin (RR) and Silver Lake (SL) were used to inoculate non-contaminated Red Cedar River sediment slurries amended with different Aroclors. The sediment slurries were then incubated under anaerobic conditions for about 9 months.

At the end of the incubation, each inoculum/Aroclor combination produced a unique product mixture of dechlorinated PCBs. The product mixtures were composed of lower chlorinated congeners than those present in the parent Aroclor. These product mixtures consisted mainly of tetra-, tri- and di-chlorobiphenyls[1]. RR- and SL-microorganisms removed 56% and 40% of the *m*- and *p*-chlorines from Aroclor 1242, and 54% and 42% of the *m*- and *p*-chlorines from Aroclor 1254, respectively. Levels of dioxin-like PCB congeners were significantly reduced, i.e., the levels of mono- and di-*ortho*-chlorinated coplanar congeners were reduced one to two orders of magnitude. Figure 1 shows the congener pattern of Aroclors 1242 and 1254 before and after dechlorination.

B. Biological Testing of Dechlorinated & Non-dechlorinated PCBs.

A battery of biological end points including *in vitro* fertilization[1], induction of AP-1 transcription activity, EROD induction[2], insulin release and neutrophil activation[3,4] were used to compare the activities of dechlorinated and non-dechlorinated PCB mixtures. For the biological assays of PCBs and their dechlorinated products, it is essential to include serum in the test medium to keep the PCBs in solution. In media containing 6% fetal bovine serum and 6% horse serum, 94% of the added PCBs remained in solution as opposed to less than 2% in the medium without serum[5].

In the *in vitro* fertilization experiments, sperm and eggs were collected from mice and exposed *in vitro* to non dechlorinated and dechlorinated PCBs along with a control which contained no PCBs. The sperm and eggs were combined in BMOC-3 medium containing PCBs and incubated. After 24 h, the fertilized eggs and degenerated eggs were counted, and the percentage of successful fertilization and egg degeneration were calculated. As shown in Figure

2, the anaerobic microbial dechlorination of PCBs significantly reduced or completely eliminated the inhibitory effects of the tested Aroclors on *in vitro* fertilization[1].

Another toxicological end point used to evaluate the effects of reductive dechlorination of PCBs was induction of the activating protein 1 transcriptional factor (AP-1 activity) which has been implicated in the neoplastic promotion process. WB-344 rat liver cell line was co-transfected with AP-1 binding DNA in front of a luciferase gene. This allowed the determination of the activity of materials which can induce the AP-1 activity. Our studies have shown that PCBs, particularly non-coplanar PCBs, are inducers of AP-1 activity. Generally the microbial dechlorination process reduced the induction of the AP-1 activity by PCBs.

The dioxin-like-activity of dechlorinated and non-dechlorinated Aroclors 1242 and 1254 was also evaluated using the ethoxyresorufin-O-deethylase (EROD) induction assays in H4IIE rat hepatoma cells. The potency of dechlorinated and non-dechlorinated PCBs as inducer of EROD activity in H4IIE cells was measured, and the biological potencies were determined from the ED50 values of full-dose response curves and expressed as tetrachloro-*p*-dioxin-equivalents (TCDD-EQs). The dechlorination process significantly reduced the TCDD-EQs of PCBs. This reduction correlated well with the reduction in the level of coplanar PCB congeners (which have strong TCDD-like activity) as a result of the microbial dechlorination.

Insulin release from the insulin producing cell line RINm5F was potentiated by the Aroclor mixtures and by non-coplanar PCB congeners[6]. In this particular assay, the microbially dechlorinated products of Aroclor 1242 and Aroclor 1254 caused equal or slightly greater (for Aroclor 1242) insulin release from RINm5F. The lower chlorinated products of PCB biodegradation appear to have the same or greater potential for causing insulin release compared to the highly chlorinated non-coplanar PCB congeners.

Induction of neutrophils to produce superoxide anion was another assay where the dechlorination of PCBs had no significant effect on potency. The ability of dechlorinated Aroclor 1242 (dechlorinated by SL and RR microorganisms) to activate rat peritoneal neutrophils and to increase the response of neutrophils to subsequent stimulation with phorbol esters was similar to that of the non-dechlorinated Aroclor. These results are consistent with previous studies demonstrating the effectiveness of *ortho*-substituted low-chlorinated PCB congeners in stimulating neutrophils to produce superoxide anion[3,4].

DISCUSSION AND CONCLUSION

Dechlorination of PCBs either caused reduction in the biological activities or, did not manifest any significant change, depending on the toxicological end point used. For example, the chlorine removal from PCB mixtures significantly lowered or completely eliminated the inhibitory effects of different Aroclors on *in vitro* fertilization, EROD induction, and induction of AP-1 activity. The same dechlorinated products demonstrated similar biological activity as the non-dechlorinated Aroclors in the cases of insulin secretion and neutrophil activation. Therefore, it appears that the microbial dechlorination of Aroclors reduces their overall toxicity. However, to accurately assess the biological activities of the products of microbial PCB dechlorination, as well as other remediation processes, a variety of toxicological end points must be used.

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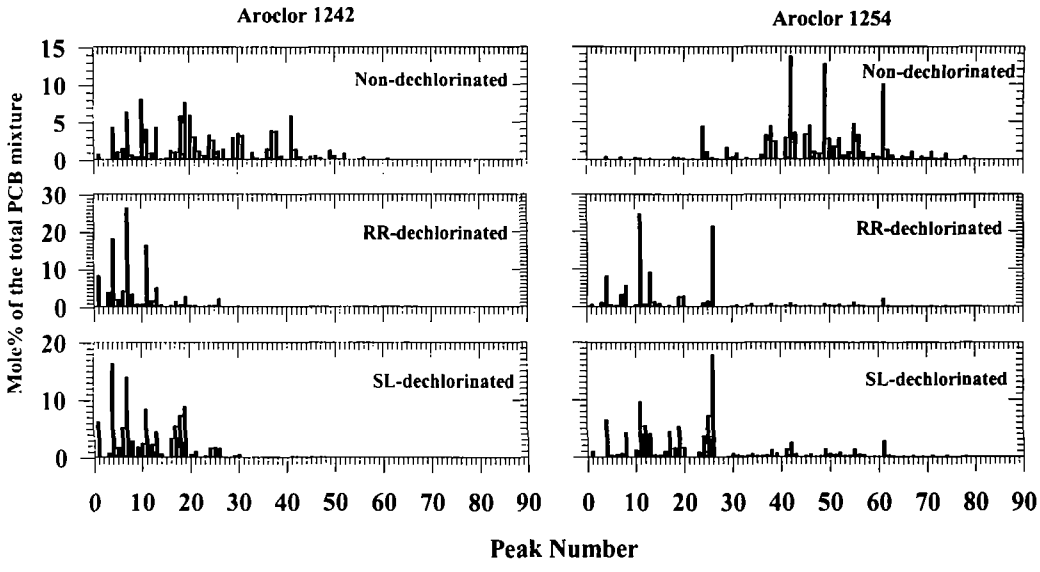

Figure 1. Chromatograms of non- and microbially-dechlorinated Aroclors 1242 & 1254[1].

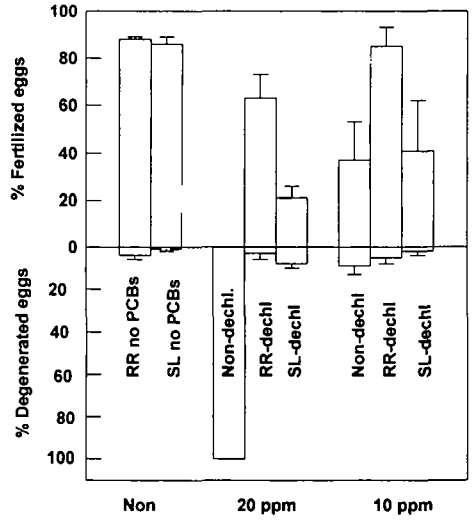

Figure 2. Dechlorination products of Aroclor 1242 are less inhibitory to *in vitro* fertilization of mouse gametes.