Antagonism of 2,3,7,8-TCDD and 3,3',4,4',5-pentaCB Induced Immunotoxicity by 2,2',4,4',5,5'-HexaCB in B6C3F1 Mice

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ABSTRACT

The toxic equivalency factor (TEF) approach has been proposed for those PCB congeners which are Ah receptor agonists. This approach assumes that the biochemical and toxic responses elicited by these compounds are essentially additive and that non-additive (synergistic/antagonistic) interactions do not play a role in the activity of PCB mixtures. This study evaluates the immunotoxicity of two Ah receptor agonists 2,3,7,8-TCDD and 3,3',4,4',5-pentaCB , and the interactive effects observed after cotreatment with the relatively non-toxic Ah receptor agonist 2,2',4,4',5,5'-hexaCB. Humoral immunotoxicity was evaluated in B6C3F1 female mice immunized with TNP-LPS using the plaque-forming cell (PFC) assay and an ELISA which detects anti-TNP IgM. 2,3,7,8-TCDD (7.2 μg/kg) decreased the PFC response and anti-TNP IgM titer to 50% and 56% of control respectively. Cotreatment of mice with 2,3,7,8-TCDD (7.2 μg/kg) and 2,2',4,4',5,5-hexaCB significantly increased the PFC response and anti-TNP IgM titer to control values. Similarly, the PFC immune response in mice treated with 3,3',4,4',5-pentaCB (12 μg/kg) was decreased to 45% of control whereas cotreatment with 2,2',4,4',5,5'-hexaCB significantly increased immune responsiveness to 73% of control. The results show that antagonistic interactions occur and should be considered when evaluating the toxicity of a complex mixture.

INTRODUCTION

Approximately 1.5 million metric tons of polychlorinated biphenyls (PCBs) have been produced throughout the world for various industrial uses.[1] Due to accidental spills or inadequate disposal of these commercial preparations, PCBs have become widespread environmental contaminants and are present in the environment as complex mixtures of biphenyls with various degrees of chlorination and different patterns of ring chlorination.[2] The use of toxic equivalency factors (TEFs) for the risk assessment of environmental PCB mixtures containing TCDD-like PCBs has been proposed, however, this approach assumes that minimal interactions between the congeners and isomers within these mixtures occur. In this study, the immunotoxic interactions between two strong Ah receptor agonists 2,3,7,8-TCDD and 3,3',4,4',5-pentaCB, and the relatively nontoxic 2,2',4,4',5,5'-hexaCB congener were investigated.

MATERIALS AND METHODS

Chemicals Sheep red blood cells (SRBCs) in Alsevars' solution were obtained from M.A. Bioproducts, Maryland, U.S.A. Guinea pig complement, RPMI 1640 media, and Earls Balanced Salt Solution (EBSS) were purchased from GIBCO Laboratories, New York, U.S.A. Trinitrophenyl lipopolysaccharide (TNP-LPS, Sigma #T4020), picryl sulfonic acid and glycyl-glycine, goat anti-mouse IgM conjugated to alkaline phosphatase (IgM-AP, #A7784), and *p*-nitrophenyl phosphate (NPP, #104) were obtained from Sigma Chemical Co. All other chemicals used were of the highest grade commercially available.

Animals B6C3F1 female mice (3 weeks) were received from an in house breeding colony and were allowed to mature to 7-10 weeks of age before treatment. All animals were maintained on a 12 hour light/dark schedule with free access to food and water. All congeners were dissolved in corn oil and administered by i.p. injection in a total volume of 10 µL/g body weight.

The PFC Assay The mice (5 per group) were treated with: corn oil, 2,3,7,8-TCDD (7.2 µg/kg); 2,3,7,8-TCDD + 2,2',4,4',5,5'-hexaCB.; or 3,3',4,4',5-pentaCB + 2,2',4,4',5,5'-hexaCB dissolved in corn oil. Two days later the mice were immunized with 50 µg of TNP-LPS in a total volume of 200 µL phosphate buffered saline (pH 7.4) by i.p. injection. Six days after exposure the mice were bled from the tail vein and terminated by cervical dislocation. The "Cunningham" modification of the Jerne plaque-forming cell (PFC) assay was used.[3,4] Trinitrophenyl haptenated SRBCs were prepared following the method of Rittenberg and Pratt.[5] The number of viable spleen cells was determined by trypan blue staining.

Detection of anti-TNP IgM by ELISA Anti-TNP IgM titer was determined by the method of Harper et al.[6] Briefly, the blood collected from each mouse was allowed to coagulate overnight at 4°C. Serum was removed from the packed red blood cells and diluted 1:10 in phosphate buffered saline (PBS pH 7.4) containing 1 % bovine serum albumin (BSA) and 0.1% sodium azide. All samples were stored at 4°C until use. Five µg of TNP-LPS in 100 uL PBS were added to each well of a 96 well plate and incubated overnight at 4°C wrapped in aluminum foil to protect from light. The plate was then washed with PBS containing 0.05% TWEEN 20 (PBS/TWEEN) and blocked with PBS containing 1% bovine serum albumin (PBS/BSA) for 1 hr at room temperature. After washing the plate with PBS/TWEEN, 100 µL of diluted serum from each animal was run in duplicate and the plates were incubated overnight at 4°C. Serum from control mice containing anti-TNP IgM from was diluted 1:400. The plate was washed with PBS/TWEEN and 100 µL of a 1:300 dilution of IgM-AP in PBS/BSA was added to each well. The plate was incubated with the secondary antibody for 2-3 hours at room temperature. In order to generate IgM units, a standard curve of IgM-AP in serial dilutions ranging from 1:300 to 1:64000 were run on each plate in duplicate. Just prior to visualization with NPP, 50 µL of each standard dilution of IgM-AP was added to each well. The ELISA was visualized by incubating 100 µL of NPP in 1M tris buffer containing 0.3 mM $MgCl_2$ (pH 9.8) at 37°C for 30 to 60 minutes. The reaction was stopped with 1 N NaOH. The plates were read on a MR600 Dynatech Microplate Reader at 410 nm. The anti-TNP-LPS IgM titer for

each mouse was quantitated after conversion to relative IgM units using the standard curve of IgM-AP vs. optical density.

RESULTS AND CONCLUSIONS

Treatment of B6C3F1 female mice with 2,2',4,4',5,5'-hexaCB (72 mg/kg) did not significantly alter spleen cellularity, the PFC response, or anti-TNP IgM titer (Table 1). Treatment with 2,3,7,8-TCDD did not alter spleen cellularity, however, the PFC response and anti-TNP IgM titer were significantly decreased to 51% and 56% of control animals respectively. Cotreatment of mice with 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexaCB significantly increased the PFC response and anti-TNP IgM in a dose-dependent manner to values similar to mice treated with vehicle only.

Table 1.
Cotreatment of female B6C3F1 mice with 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexaCB, immunized with TNP-LPS.

Cotreat with	Dose $(245)^2$-HCB mg/kg	Cells/ Spleen $10^8 \pm$ S.E.	Plaques/ Spleen $10^5 \pm$ S.E.	PFC/1E6 Cells ± S.E.	IgM Units ± S.E.
	control	1.68 ± 0.12	2.88 ± 0.22	1821 ± 175	1368 ± 124
	72	1.80 ± 0.14	2.76 ± 0.38	1625 ± 154	1282 ± 182
2,3,7,8-	control	1.83 ± 0.19	2.98 ± 0.15	1699 ± 192	2790 ± 60
TCDD	0	1.28 ± 0.06	1.48 ± 0.15*	1151 ± 67*	1590 ± 45*
7.2 μg/kg	18	1.21 ± 0.06	1.60 ± 0.06*	1332 ± 88	---
	36	1.25 ± 0.07	2.84 ± 0.08[a]	2293 ± 128[a]	2800 ± 38[a]
	72	1.36 ± 0.12	3.25 ± 0.11[a]	2480 ± 270[a]	—

* $p<0.05$ from control, determined by ANOVA with Duncan Multiple Range post hoc

[a] $p<0.05$ from 2,3,7,8-TCDD, determined by ANOVA with Duncan Multiple Range post hoc

Similarly, in mice exposed to the TCDD-like PCB congener, 3,3',4,4',5-pentaCB (12 μg/kg), the PFC response and IgM titer were significantly decreased to less than 50% of control animals while spleen cellularity was not affected (Table 2). Cotreatment of mice with 3,3',4,4',5-pentaCB and the 2,2',4,4',5,5'-hexaCB congener increased immune responsiveness in a dose-dependent manner to values that were not significantly different from mice treated with the vehicle only.

The results show that 2,3,7,8-TCDD induced immunotoxicity is antagonized by 2,2',4,4',5,5'-hexaCB in B6C3F1 mice immunized with the T-cell independent antigen TNP-LPS. These results are similar to those observed by Biegel and coworkers in which 2,2',4,4',5,5'-hexaCB inhibited 2,3,7,8-TCDD induced immunosuppression in C57BL/6 mice immunized with sheep red blood cells; a T-cell dependent antigen.[7] Moreover, immunosuppression caused by 3,3',4,4',5-pentaCB was antagonized by the relatively nontoxic congener 2,2',4,4',5,5'-hexaCB. These non-additive interactions suggest that the TEF approach for risk estimation of PCB mixtures may be highly conservative when the

sample contains PCBs such as 2,2',4,4',5,5'-hexaCB which appear to act as Ah receptor antagonists.

Table 2.
Cotreatment of female B6C3F1 mice with 3,3',4,4',5-pentaCB and 2,2',4,4',5,5'-HCB immunized with TNP-LPS.

Cotreat with	Dose $(245)^2$-HCB mg/kg	Cells/ Spleen $10^8 \pm$ S.E.	Plaques/ Spleen $10^5 \pm$ S.E.	PFC/1E6 Cells ± S.E.	IgM Units ± S.E.
	control	1.68 ± 0.12	2.88 ± 0.22	1821 ± 175	1368 ± 124
	72	1.80 ± 0.14	2.76 ± 0.38	1625 ± 154	1282 ± 182
3,3',4,4',5-	control	1.80 ± 0.17	3.55 ± 154	1984 ± 154	903 ± 52
pentaCB	0	1.73 ± 0.05	1.48 ± 0.05*	862 ± 49*	381 ± 25*
(12 µg/kg)	9	1.72 ± 0.25	1.86 ± 0.16*	1186 ± 220*	610 ± 69*
	36	2.02 ± 0.20	2.76 ± 0.18[b]	1403 ± 157[b]	844 ± 23[b]
	72	2.17 ± 0.24	2.98 ± 0.15[b]	1443 ± 164[b]	835 ± 33[b]

* $p<0.05$ from control, determined by ANOVA with Duncan Multiple Range post hoc
[b] $p<0.05$ from 3,3',4,4',5-pentaCB, determined by ANOVA with Duncan Multiple Range post hoc

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