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**AROCLOR 1254 AS A 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN
ANTAGONIST: EFFECTS ON ENZYME INDUCTION AND
IMMUNOTOXICITY**

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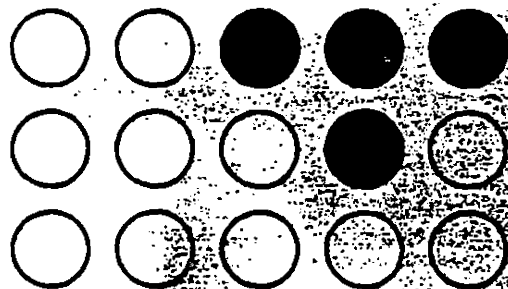
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AROCOR 1254 AS A 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ANTAGONIST: EFFECTS ON ENZYME INDUCTION AND IMMUNOTOXICITY

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SUMMARY

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Aroclor 1254 induced the cytochrome *P*-450 dependent monooxygenases, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) in rat hepatoma H-4-II E cells and C57BL/6J mice. It has been proposed that both Aroclor 1254 and 2,3,7,8-TCDD induce these enzymes via a common mechanism which features initial binding to the aryl hydrocarbon (Ah) cytosolic receptor protein. The major difference between these compounds was the relative potency (i.e. 2,3,7,8-TCDD $\gg$ Aroclor 1254). Cotreatment of rat hepatoma H-4-II E cells or C57BL/6J mice with a dose of 2,3,7,8-TCDD which submaximally induces AHH and EROD and a dose of Aroclor 1254 which exhibited little or no induction activity resulted in significant antagonism of the induction effects of 2,3,7,8-TCDD. For example, cotreatment of C57BL/6J mice with 2,3,7,8-TCDD (15 nmol/kg) and Aroclor 1254 (25, 75 and 150 μ mol/kg) resulted in up to 23% antagonism of AHH induction by 2,3,7,8-TCDD. Moreover, cotreatment with a higher dose of the 2,3,7,8-TCDD agonist (30 or 50 nmol/kg) partially reversed some of the antagonism by Aroclor 1254. In vivo antagonism was observed only at Aroclor 1254/2,3,7,8-TCDD molar ratios of 1667:1, 5000:1 and 10 000:1. Administration of 2,3,7,8-TCDD (3.72 nmol/kg) to C57BL/6J mice resulted in a 76% decrease in the splenic plaque forming cell response to sheep red blood cells. This T-cell mediated immunotoxic effect of 2,3,7,8-TCDD segregates with

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Abbreviations: Ah, aryl hydrocarbon; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; HCBP, hexachlorobiphenyl; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; SARs, structure-activity relationships; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran.

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tionships (SARs) have recently been reported for several structurally diverse PCDDs, PCDFs and PCBs [9-11,20]. Comparable SARs were observed for cytosolic receptor binding, the *in vitro* induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD), the *in vivo* induction of these same monooxygenase enzymes, body weight loss and thymic atrophy (in the rat).

Previous studies with agonists for the estrogen receptor have demonstrated that some compounds which exhibit moderately high estrogen receptor binding affinities but low estrogenic activities (e.g. estriol and some triphenylethylene analogs) can antagonize the effects of estradiol [21,22]. Similarly, recent studies in our laboratory have shown that both 1,3,6,8- and 2,4,6,8-tetrachlorodibenzofuran (TCDF) exhibit relatively high cytosolic receptor binding affinities (1.25×10^{-7} M and 1.5×10^{-8} M, respectively) but are only weakly active as inducers of AHH and EROD in rat hepatoma H-4-II E cells in culture [23]. Cotreatment of the cells with 2,3,7,8-TCDD and either of the 2 PCDF isomers demonstrated that both compounds antagonized the induction of the monooxygenases by 2,3,7,8-TCDD. Previous studies [8] have reported that the commercial PCB, Aroclor 1254 also exhibited moderate binding affinity to the cytosolic receptor protein (6.6×10^{-6} M) but was a weak agonist for most receptor-mediated responses *in vivo* and *in vitro* [24]. This paper reports the activity of Aroclor 1254 as a 2,3,7,8-TCDD antagonist in rat hepatoma H-4-II E cells and in C57BL/6 mice.

MATERIALS AND METHODS

Chemicals

2,3,7,8-TCDD and ethoxyresorufin were synthesized in this laboratory to greater than 99% purity as determined by gas chromatography [10]; Aroclor 1254 was a gift from Dr. O. Hutzinger; NADPH and NADH were purchased from the United States Biochemical Corporation (Cleveland, OH). Benzo[a]pyrene was purchased from Sigma Chemical Co.; DNA-grade hydroxylapatite was purchased from Bio-Rad Laboratories (Richmond, CA). All other chemicals were of the highest quality commercially available. [3 H]2,3,7,8-TCDD was synthesized in the laboratory by the chlorination of 1,6-[3 H]₂dibenzo-*p*-dioxin followed by chlorination and HPLC purification of the product (>98% pure) [5].

In vivo enzyme induction studies

Male C57BL/6J, 3-4 weeks of age, were obtained from the Jackson Laboratories, Bar Harbor, ME. The mice were housed in plastic cages with hardwood bedding, allowed free access to Teklab Laboratory Rodent Chow, (Teklab, Madison, WI) and water and maintained on a diurnal 12-h light/12-h dark cycle. Aroclor 1254 or 2,3,7,8-TCDD were dissolved in corn oil and administered by intraperitoneal injection (10 ml/kg). The C57BL/6J mice treated with Aroclor 1254 and/or 2,3,7,8-TCDD were sacrificed after 72 h.

Corn oil (10 ml/kg) served as a vehicle control for all studies. Livers were perfused via the hepatic portal vein with ice cold saline containing 0.1 mM EDTA, weighed and placed in beakers containing homogenization buffer. Livers were washed and then homogenized using a Potter-Elvehjem homogenizer in 4 volumes of 0.25 M sucrose with 0.1 mM EDTA. A microsomal pellet was prepared by centrifuging the homogenate at 10 000 g for 20 min and then centrifuging the resultant supernatant at 100 000 g for 1 h. The pellet was resuspended in homogenization buffer and stored at -80°C until used for the enzyme assays. Ethoxyresorufin *o*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) were determined as previously described [25,26]. Protein concentrations were determined [27] using bovine serum albumin as a protein standard. All data are expressed as means $\pm$ S.D. Significance was determined by a two-way ANOVA and Dunnet's *t*-test [28].

In vitro enzyme induction

Rat hepatoma H-4-II E cells were grown in minimum essential medium without ribo- or deoxyribonucleotides but with L-glutamine. The medium was supplemented with 10% fetal calf serum, 10% calf serum, gentamycin sulfate (50 $\mu\text{g}/\text{ml}$) and Fungizone (2.5 $\mu\text{g}/\text{ml}$) and cultures were maintained in a humidified 5% CO_2 atmosphere at 37°C . At confluency stock cultures were trypsinized and seeded in 25-cm² Corning culture flasks at a density of 0.6×10^6 cells/flask (Day 1). On Day 2, the spent medium was aspirated off and replaced with fresh medium containing the test compound in DMSO to a final DMSO concentration of 0.5%. Dose-response induction studies with at least 6 different concentrations of each inducer were carried out. On Day 3, the cultures reached confluency and were harvested. Cultures were washed 3 times with PBS (pH 7.4) and scraped off with a rubber policeman in Tris-sucrose (0.05–0.02 M) buffer (pH 8.0). The cell suspensions were centrifuged and resuspended in buffer. After protein determination [27], the cell suspensions were adjusted to a final protein concentration of 1 mg/ml and whole cell suspensions were assayed for enzyme activity. EROD and AHH activities were measured fluorimetrically [25,26] and the EC_{50} values determined as previously described [9–11].

Receptor binding assays

Hepatic cytosol (~3–5 mg/ml) was incubated with different concentrations of [³H]2,3,7,8-TCDD and Aroclor 1254 (100, 500 and 667 nM) for 2 h at 20°C . Each assay was performed in duplicate. A hydroxylapatite slurry (Bio Rad Lab; 3 parts gel in 5 parts phosphate or HEDG buffer) was freshly prepared. The phosphate buffer was 50 mM Tris-HCl, 1 mM KH_2PO_4 . After incubation, 200 μl aliquot of the cytosol was transferred to a second tube containing 250 μl of the hydroxylapatite slurry. The tubes were incubated on ice for 30 min with shaking every 10 min. The mixture was then resuspended in 2 ml phosphate buffer and centrifuged at 800 g for 2 min. The hydroxylapatite pellets were washed 3 additional times with phosphate buffer (2.0 ml buffer) and then resuspended in 1.0 ml absolute ethanol. The

hydroxylapatite-ethanol solution was vortexed and transferred to scintillation vials and any remaining hydroxylapatite was washed into the vial with an additional 1 ml of ethanol; 10 ml of scintillation cocktail was added and samples were counted for radioactivity. Saturation binding of [³H]2,3,7,8-TCDD with C57BL/6J hepatic cytosolic receptor protein was determined in the presence of different concentrations of the competitive ligand, Aroclor 1254 and the results are summarized in Fig. 2.

Plaque forming cell (PFC) assay

The method employed was the "Cunningham" variant of the Jerne PFC assay [29,30]. C57BL/6J mice at 8 weeks were purchased from the Jackson Laboratories, Bar Harbor, ME. Upon arrival the animals were allowed to acclimate for 5-7 days before treatment. On day 1 the animals were injected (i.p.) with the appropriate chemical as described for the enzyme induction studies (Aroclor 1254 and/or 2,3,7,8-TCDD) and sheep red blood cells (SRBC, 4×10^8 cells) were injected after 5 days. The animals were sacrificed by cervical dislocation 10 days after treatment with the chemicals and the spleens were removed and placed individually in 60-mm petri dishes containing 10 ml of cold Eagle's MEM media supplemented with Hank's salts (KC Biological). Each spleen was then teased through a 70- μ m nylon screen (Spectrum Medical, Inc.) which was saturated with 2 ml of the Eagle's media. The screens were washed of remaining cells by the addition of 8 ml of media. The cell solution was then spun at 220 g for 10 min and the pellets resuspended in 2 ml of fresh media. Appropriate dilutions of the suspensions were then made with 0.1 ml of a dilution mixed with 0.2 ml of a 20% SRBC solution and 0.1 ml of a 1-5 guinea pig serum dilution. Through capillary action, microliter aliquots of the mixtures were placed in "Cunningham" slide chambers and incubated for 60 min at 37°C. Viable cell counts were determined by Eosin Y staining.

RESULTS

2,3,7,8-TCDD evoked a dose-dependent induction of AHH and EROD in rat hepatoma H-4-II E cells at concentrations of 10^{-12} – 10^{-8} M. At higher dose levels ($>10^{-8}$ M), 2,3,7,8-TCDD was toxic to the cells and this was evidenced by a dose-dependent decrease in the AHH induction maximum with increasing concentrations of 2,3,7,8-TCDD (Fig. 1). The dose-dependent increase and subsequent decrease of AHH and EROD induction has previously been reported for other halogenated aromatics [24]. Moreover, for some weak agonists such as Aroclor 1254, cellular toxicity is noted at concentrations which are insufficient to elicit maximum induction of the cytochrome P-450-dependent monooxygenases (i.e. see Fig. 1). Cotreatment of the rat hepatoma cells with a non-toxic concentration of Aroclor 1254

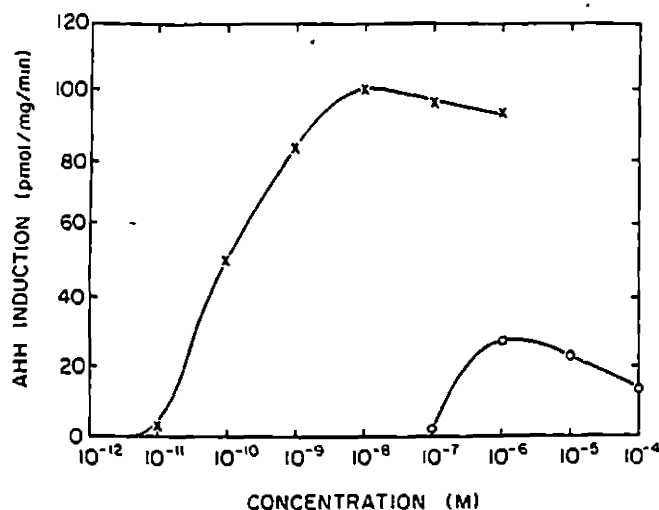


Fig. 1. Dose response induction of AHH (pmol/mg protein/min) in rat hepatoma H-4-II E cells by Aroclor 1254 (O) and 2,3,7,8-TCDD (X). Cellular toxicity by Aroclor 1254 and 2,3,7,8-TCDD was observed at concentrations $> 10^{-6}$ M and 10^{-8} M, respectively.

(1×10^{-7} M) and a concentration of 2,3,7,8-TCDD which elicited a submaximal AHH and EROD induction response (1×10^{-9} M) resulted in the antagonism of the induction activity of 2,3,7,8-TCDD by Aroclor 1254 (Table I). Cotreatment of the cells with the same concentration of Aroclor 1254 (1×10^{-7} M) and a higher concentration of 2,3,7,8-TCDD (2.25×10^{-8} M) par-

TABLE I

EFFECTS OF 2,3,7,8-TCDD, AROCLOR 1254 AND 2,3,7,8-TCDD PLUS AROCLOR 1254 (COTREATED) AS INDUCERS OF AHH AND EROD IN RAT HEPATOMA H-4-II E CELLS IN CULTURE

Inducer	Concentration (M)	EROD ^b	AHH ^b
Solvent (control)	—	0	0
Aroclor 1254	1×10^{-7}	0	0
2,3,7,8-TCDD	1×10^{-9}	74.0 ± 20.1	155.9 ± 14.6
2,3,7,8-TCDD	2.25×10^{-8}	77.9 ± 19.9	179.0 ± 19.7
Aroclor 1254 plus	1×10^{-7}	46.8 ± 3.99^a	105.7 ± 11.7^a
2,3,7,8-TCDD	1×10^{-9}		
Aroclor 1254 plus	1×10^{-7}	65.6 ± 2.21	117.0 ± 7.36
2,3,7,8-TCDD	2.25×10^{-8}		

^aSignificantly lower ($P < 0.01$) than observed after treatment with 2,3,7,8-TCDD (1.0×10^{-9} M) alone.

^bpmol/mg protein/min.

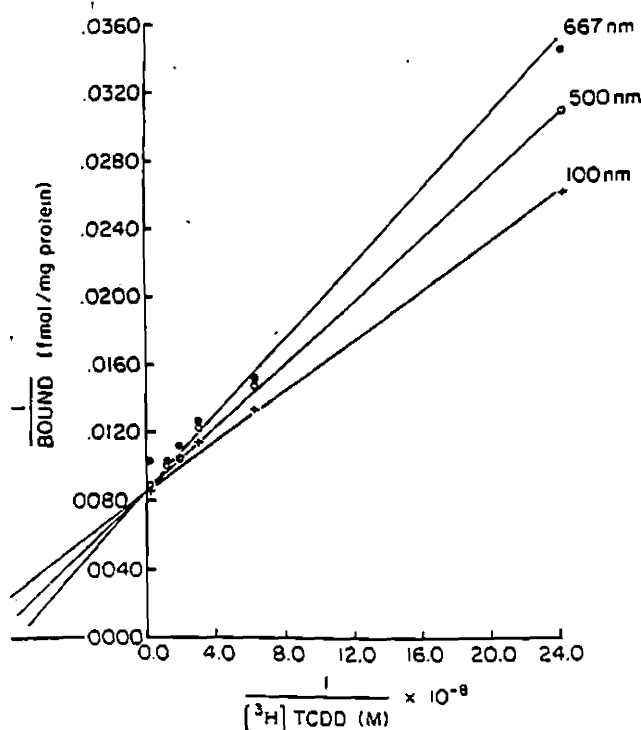


Fig. 2. Double reciprocal plot analysis of the saturation binding of [³H]2,3,7,8-TCDD in the presence of different concentrations of Aroclor 1254 (100, 500 and 667 nM). The y intercepts of the double-reciprocal plots of the saturation binding of [³H]2,3,7,8-TCDD with the Ah receptor in the presence of 100, 500 and 667 nM Aroclor 1254 were 0.0091, 0.0088 and 0.0092, respectively. The linear correlation coefficients were 0.9925, 0.9984 and 0.9995, respectively. In the absence of Aroclor 1254, the y intercept of the double-reciprocal plot of the saturation binding of [³H]2,3,7,8-TCDD with the Ah receptor was 0.0074 and the correlation linear correlation coefficient was 0.9899 (data not shown). The incubations were carried out using hepatic cytosol preparation (3–5 mg/ml) and a range of [³H]2,3,7,8-TCDD concentrations (from 0.8 to 11 nM).

tially overcame the antagonism by Aroclor 1254. Double reciprocal plot analysis of the saturation binding curves of [³H]2,3,7,8-TCDD and rat hepatic cytosolic receptor in the presence of different concentrations of Aroclor 1254 is summarized in Fig. 2 and demonstrates that Aroclor 1254 acts as a competitive antagonist for the receptor-2,3,7,8-TCDD interaction.

Table II summarizes the dose-response effects of 2,3,7,8-TCDD and Aroclor 1254 as inducers of hepatic microsomal AHH and EROD in immature male C57BL/6J mice. Maximal induction of AHH (4.06 nmol/mg/min) and EROD (4.86 nmol/mg/min) were observed at a dose level of 50 nmol/kg of 2,3,7,8-TCDD. No overt toxicity (e.g. body weight loss, hepatotoxicity or thymic atrophy) was observed at this dose level. In contrast, administration of Aroclor 1254 to C57BL/6J mice at doses of 5, 25, 75, 150 and 400 μmol/kg significantly induced EROD only at the highest dose of Aroclor 1254

TABLE II

THE DOSE-RESPONSE EFFECTS OF 2,3,7,8-TCDD AND AROCLOR 1254 AS INDUCERS OF HEPATIC MICROSOMAL AHH AND EROD IN MALE C57BL/6J MICE (n = 5)

Compound	Dose ($\mu\text{mol/kg}$)	EROD ^b	AHH ^b
Corn oil	—	0.06 $\pm$ 0.004	0.21 $\pm$ 0.08
2,3,7,8-TCDD	0.001	0.33 $\pm$ 0.08 ^a	0.64 $\pm$ 0.16 ^a
2,3,7,8-TCDD	0.003	1.39 $\pm$ 0.54 ^a	1.67 $\pm$ 0.71 ^a
2,3,7,8-TCDD	0.010	3.31 $\pm$ 0.50 ^a	3.36 $\pm$ 0.07 ^a
2,3,7,8-TCDD	0.015	3.96 $\pm$ 0.16 ^a	3.56 $\pm$ 0.43 ^a
2,3,7,8-TCDD	0.03	4.19 $\pm$ 0.30 ^a	3.67 $\pm$ 0.30 ^a
2,3,7,8-TCDD	0.05	4.86 $\pm$ 0.61 ^a	4.06 $\pm$ 0.37 ^a
Aroclor 1254	5	0.06 $\pm$ 0.006	0.18 $\pm$ 0.06
Aroclor 1254	25	0.05 $\pm$ 0.03	0.41 $\pm$ 0.06 ^a
Aroclor 1254	75	0.06 $\pm$ 0.01	0.39 $\pm$ 0.06 ^a
Aroclor 1254	150	0.09 $\pm$ 0.04	0.40 $\pm$ 0.08 ^a
Aroclor 1254	400	0.25 $\pm$ 0.05 ^a	0.55 $\pm$ 0.07 ^a

^aSignificantly different ($P < 0.01$) from the corn oil controls.

^bnmol/mg protein/min.

TABLE III

COTREATMENT OF C57BL/6J MICE WITH AROCLOR 1254 AND 2,3,7,8-TCDD: EFFECTS ON AHH AND EROD INDUCTION (n = 5)

Inducers	Dose ($\mu\text{mol/kg}$)	EROD ^c	AHH ^c
Aroclor 1254 plus 2,3,7,8-TCDD	5 0.015	3.79 $\pm$ 0.58	3.34 $\pm$ 0.42
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.015	3.05 $\pm$ 0.47 ^a	2.84 $\pm$ 0.33 ^a
Aroclor 1254 plus 2,3,7,8-TCDD	75 0.015	3.09 $\pm$ 0.34 ^a	2.88 $\pm$ 0.21 ^a
Aroclor 1254 plus 2,3,7,8-TCDD	150 0.015	3.22 $\pm$ 0.07 ^b	3.09 $\pm$ 0.27 ^a
Aroclor 1254 plus 2,3,7,8-TCDD	400 0.015	3.96 $\pm$ 0.45	3.55 $\pm$ 0.18
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.03	4.19 $\pm$ 0.39	3.78 $\pm$ 0.25
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.05	4.93 $\pm$ 0.25	4.10 $\pm$ 0.20

Significantly lower ^a($P < 0.01$) ^b($P < 0.05$) than induction values observed after treatment with 2,3,7,8-TCDD (15 nmol/kg) alone; the induction values for 2,3,7,8-TCDD (0.015, 0.03 and 0.5 $\mu\text{mol/kg}$) are shown in Table II.

^cnmol/mg protein/min.

and marginally increased AHH at doses above 25 $\mu\text{mol/kg}$. Table III summarizes the effects of coadministration of Aroclor 1254 (5, 25, 75, 150 or 400 $\mu\text{mol/kg}$) with a dose of 2,3,7,8-TCDD (15 nmol/kg) which elicited a sub-maximal AHH and EROD induction response. Cotreatment of 2,3,7,8-TCDD with 25 or 75 $\mu\text{mol/kg}$ of Aroclor significantly inhibited the induction of AHH and EROD by 2,3,7,8-TCDD. Coadministration of Aroclor 1254 (25 $\mu\text{mol/kg}$) with higher doses of 2,3,7,8-TCDD (30 and 50 nmol/kg) reversed the antagonistic effects of Aroclor 1254.

Table IV summarizes the effects of 2,3,7,8-TCDD and Aroclor 1254 on the T-cell dependent plaque-forming cell (PFC) assay after exposure of

TABLE IV

EFFECTS OF 2,3,7,8-TCDD, AROCLOR 1254 AND 2,3,7,8-TCDD PLUS AROCLOR 1254 ON THE PLAQUE FORMING CELL ASSAY IN MICE PRETREATED WITH SHEEP RED BLOOD CELLS

Treatment (dose)	PFC/spleen $\times 10^5$	PFC/ 10^6 viable spleen cells	% Control (PFC/spleen $\times 10^5$)
Corn oil ^a	1.61 ± 0.17	562 ± 76	100
2,3,7,8-TCDD (3.72 nmol/kg) ^b	0.38 ± 0.03^c	156 ± 40^c	24
(11.2 nmol/kg)	0.20 ± 0.03^c	62 ± 12^c	12
Aroclor 1254 (5 $\mu\text{mol/kg}$)	1.57 ± 0.11	561 ± 140	98
(15 $\mu\text{mol/kg}$) ^b	1.60 ± 0.87	465 ± 114	100
(75 $\mu\text{mol/kg}$)	1.57 ± 0.23	594 ± 35	98
(150 $\mu\text{mol/kg}$)	1.20 ± 0.22	394 ± 35	75
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (5 $\mu\text{mol/kg}$)	1.70 ± 0.30	491 ± 62	106
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (15 $\mu\text{mol/kg}$) ^b	1.49 ± 0.08	480 ± 48	93
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (75 $\mu\text{mol/kg}$)	1.66 ± 0.28	558 ± 52	103
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (150 $\mu\text{mol/kg}$)	0.92 ± 0.08^c	372 ± 81^c	57
2,3,7,8-TCDD (11.2 nmol/kg) plus Aroclor 1254 (75 $\mu\text{mol/kg}$)	0.58 ± 0.10^c	170 ± 10^c	36

^a11 animals.

^b8 animals; all other 4 animals/group.

^cSignificantly different ($P < 0.01$) than the corn oil controls.

C57BL/6J mice to sheep red blood cells. Administration of 2,3,7,8-TCDD (3.72 and 11.2 nmol/kg) resulted in only 24 and 12% PFCs/ 10^6 spleen cells respectively, compared to the corn oil-treated animals. In contrast, administration of Aroclor 1254 at dose levels of 5, 15, and 75 μ mol/kg did not significantly impair the immune response although administration of a higher dose of Aroclor 1254 (150 μ mol/kg) did evoke some decrease in the plaque forming cell response. Cotreatment of the mice with 2,3,7,8-TCDD (3.72 nmol/kg) and 5, 15, or 75 μ mol/kg Aroclor 1254 resulted in significant protection of these animals from the immunotoxic effects of 2,3,7,8-TCDD (Table IV). Significant antagonism of 2,3,7,8-TCDD was also observed in animals cotreated with the highest dose of Aroclor 1254 (150 μ mol/kg) which was also immunotoxic in this assay when administered alone. Coadministration of a higher dose of 2,3,7,8-TCDD (11.2 nmol/kg) with Aroclor 1254 (75 μ mol/kg) reversed some of the antagonistic effects of the commercial PCB mixture.

DISCUSSION

This study describes the in vitro and in vivo interactive effects of 2,3,7,8-TCDD and Aroclor 1254, 2 compounds which individually elicit a number of common biologic and toxic effects in diverse animal species. For example, both of these halogenated aryl hydrocarbons induce the same monooxygenase enzymes (e.g. AHH and EROD) and their associated cytochrome P-450 isozymes, cause body weight loss, hepatotoxicity and porphyria, thymic atrophy and immunotoxicity [1-3,24]. In addition, 2,3,7,8-TCDD and Aroclor 1254 competitively bind to the Ah or 2,3,7,8-TCDD receptor protein [8]. These results support the proposed common mechanism of action for both compounds; however, it is apparent from this study and others that the major differences between Aroclor 1254 and 2,3,7,8-TCDD are their toxic and biologic potencies. The results summarized in Tables I and II clearly illustrate the quantitative differences between 2,3,7,8-TCDD and Aroclor 1254 as inducers of AHH and EROD. In rat hepatoma cells, the EC_{50} for 2,3,7,8-TCDD as an inducer of AHH and EROD was 9.78×10^{-11} and 1.90×10^{-10} M respectively whereas the EC_{50} for Aroclor 1254 as an inducer of these enzymes was $> 10^{-6}$ M. Moreover in C57BL/6J mice, 2,3,7,8-TCDD was $> 50\,000$ times more potent than Aroclor 1254 as an inducer of hepatic microsomal AHH and EROD. 2,3,7,8-TCDD and Aroclor 1254 and several PCB congeners are immunotoxic and inhibit the plaque forming antibody response in C57BL/6J mice challenged with sheep erythrocytes [13-17,31]. The ED_{50} for inhibition of this response by Aroclor 1254 in C57BL/6N mice was between 250 and 500 mg/kg [31] whereas the ED_{50} for 2,3,7,8-TCDD was $< 1.2 \mu$ g/kg [14].

Previous in vitro studies have reported that 1,3,6,8- and 2,4,6,8-tetrachlorodibenzofuran antagonize the induction of AHH and EROD in rat hepatoma H-4-II E cells in culture [23]. Like Aroclor 1254, both of these isomers exhibit moderate competitive receptor binding affinities and are weak

inducers of AHH and EROD in the cell culture system. Table I summarizes the interactive effects of 2,3,7,8-TCDD and Aroclor 1254 in the rat hepatoma H-4-II E system and at Aroclor 1254/2,3,7,8-TCDD concentration ratios of 100:1 there was a significant inhibition of the induction activity of 2,3,7,8-TCDD by Aroclor 1254. Moreover at a higher dose of the agonist (2.25×10^{-6} M) some of the inhibitory effects of Aroclor 1254 were overcome. A comparable interaction study was carried out in C57BL/6J mice. The mice were cotreated with a dose of 2,3,7,8-TCDD which elicited a sub-maximal induction response for hepatic microsomal AHH and EROD (15 nmol/kg) and one of several doses of Aroclor 1254. The results showed the Aroclor significantly reduced the induction of AHH and EROD by 2,3,7,8-TCDD (15 nmol/kg) only at doses of 25, 75 and 150 μ mol/kg. The role of Aroclor 1254 as a 2,3,7,8-TCDD antagonist was further supported by the effects of cotreating the animals with the most effective "inhibitory" dose of Aroclor 1254 (25 μ mol/kg) and higher doses of 2,3,7,8-TCDD (30 and 50 nmol/kg) (Table III). These results showed that at higher dose levels of the agonist the partial antagonist activity of Aroclor 1254 was decreased complementing the results obtained in the in vitro studies. It was also apparent that Aroclor 1254 was a more effective partial antagonist of the induction responses by 2,3,7,8-TCDD in cell culture. Significant antagonism of AHH was observed at Aroclor 1254/2,3,7,8-TCDD ratios of 100:1 in cell cultures whereas antagonism was only observed in the mice at ratios between 1667:1 and 10 000:1.

Several reports have shown that 2,3,7,8-TCDD and related compounds are immunotoxic and impair many T-cell mediated functions [13-17,31-34]. Moreover the T-cell dependent plaque forming cell response to sheep red blood cells is one of the most sensitive indicators of exposure to toxic halogenated aryl hydrocarbons and studies in genetically inbred mice have provided strong evidence that this response is Ah receptor-mediated. Table IV summarizes the immunosuppressive effects of 2,3,7,8-TCDD and Aroclor 1254 in C57BL/6J mice using the plaque forming cell assay response to sheep red blood cells. 2,3,7,8-TCDD was highly immunotoxic whereas doses of 5, 15 and 75 μ mol/kg of Aroclor 1254 were inactive in this assay system. This latter result was not surprising since Aroclor 1254 was previously reported to be inactive at doses of < 125 mg/kg (382 μ mol/kg) [31]. Cotreatment of the mice with 2,3,7,8-TCDD (3.72 nmol/kg) and different doses of Aroclor 1254 (5, 15 or 75 μ mol/kg) resulted in complete protection from the immunotoxic effects of 2,3,7,8-TCDD by Aroclor 1254 (5, 15 or 75 μ mol/kg). Moreover, the antagonistic effects of Aroclor 1254 (75 μ mol/kg) can be partially overcome by a higher dose of the 2,3,7,8-TCDD agonist (11.2 nmol/kg).

The interactive effects of 2,3,7,8-TCDD, a potent Ah receptor agonist, and Aroclor 1254 suggest that this commercial PCB mixture can antagonize at least 2 Ah receptor mediated responses, namely immunotoxicity and the induction of cytochrome P_1 -450-dependent monooxygenases (AHH and EROD). These effects are dependent on the dose of both the antagonist and

agonist and their respective antagonist/agonist ratios. For antagonism of AHH and EROD induction by 2,3,7,8-TCDD in C57BL/6J mice, an Aroclor 1254/2,3,7,8-TCDD ratio of 1667:1 gave the maximum antagonism (23%) of the EROD induction response. In contrast, Aroclor 1254/2,3,7,8-TCDD ratios from 1340:1 to 20 160:1 resulted in complete protection from the immunotoxic effect of 2,3,7,8-TCDD (3.72 nmol/kg). The results demonstrate that the "window of antagonism" for Aroclor 1254 is clearly dependent not only on the absolute and relative doses of Aroclor 1254 and 2,3,7,8-TCDD but on the specific receptor-mediated response which is being measured. However, it was observed that for Aroclor 1254/2,3,7,8-TCDD ratios which result in antagonism by Aroclor 1254 of AHH and EROD induction (in vivo and in vitro) and immunotoxicity, an increased dose of 2,3,7,8-TCDD overcame some of the partial antagonism by Aroclor 1254. These results, coupled with the double-reciprocal plot analysis of the in vitro binding data (Fig. 2) suggest that the antagonist activity of Aroclor 1254 is related to the competitive inhibition of 2,3,7,8-TCDD binding to the Ah receptor and is not due to cellular toxicity caused by Aroclor 1254.

A previous study has reported that 2,3-dimethyl-5-*t*-butyl-1,3-benzodioxole (DBBD) inhibits EROD induction by 3-methylcholanthrene [35]. However, it is unlikely that the mechanism of antagonism for this compound involves competitive inhibition of receptor binding since DBBD exhibits a low binding affinity for the Ah receptor [36]. A more recent study suggested that the activity of DBBD may be associated with down regulation of the Ah receptor [37]. A recent paper [38] reported that 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin was also an effective antagonist for 2,3,7,8-TCDD induced myelotoxicity (in vivo) and enzyme induction (in vitro). They also proposed that this compound acts as a competitive antagonist and their results complement the data reported in this study which demonstrates that Aroclor 1254 antagonizes 2,3,7,8-TCDD-mediated enzyme induction and immunotoxicity in C57BL/6J mice.

It is interesting to note that the relatively high ratios of Aroclor 1254/2,3,7,8-TCDD which result in antagonist activity are comparable to the ratios of PCBs/PCDFs or PCBs/PCDDs which have been identified in the analysis of human tissues and environmental samples [39,40]. It is conceivable that environmental or accidental exposure to these mixtures within certain exposure levels may result in some protection from the highly toxic PCDDs and PCDFs by PCBs and this possibility is currently being investigated in our laboratory.

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