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AROCLOR 1254 AS AN ANTAGONIST OF THE TERATOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

(Aroclor 1254; 2,3,7,8-TCDD; antagonism)

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SUMMARY

Administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 20 µg/kg) to pregnant C57BL/6J mice (on day 10) resulted in 62% fetuses with cleft palate per litter without any observable maternal toxicity. In contrast, Aroclor 1254 administered at a dose of 750 µmol/kg was not teratogenic. Cotreatment of the pregnant mice with both Aroclor 1254 (244 mg/kg) and 2,3,7,8-TCDD (20 µg/kg) resulted in an 8.2% incidence of cleft palate per litter. In contrast, Aroclor 1254 did not afford any protection from the teratogenicity of dexamethasone in C57BL/6J mice. Previous studies have shown that Aroclor 1254 can act as a partial antagonist of the microsomal enzyme induction and immunotoxic effects of 2,3,7,8-TCDD in C57BL/6J mice and this paper demonstrates that the commercial polychlorinated biphenyl mixture also antagonizes 2,3,7,8-TCDD-mediated teratogenicity in this strain of mice.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are widely used industrial compounds which have been identified in almost every component of the global ecosystem including fish, wildlife and human adipose tissue, blood and milk [1-4]. During the past few years, the more toxic halogenated aryl hydrocarbons, namely the polychlorinated

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Abbreviations: Ah, aryl hydrocarbon; AHH, aryl hydrocarbon hydroxylase; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; TCDD, tetrachlorodibenzo-*p*-dioxin.

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dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) have also been identified in the environment [5-8] and in human tissue including milk samples [9-16]. Since the PCDFs and PCDDs are industrial or combustion byproducts [17-19], the levels of these compounds are usually $>10^4$ -fold lower in most environmental samples. Moreover, high resolution analyses of the human milk and adipose tissue have confirmed the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic halogenated aryl hydrocarbon and several other toxic 2,3,7,8-substituted PCDDs and PCDFs [9-16].

The toxicity and mechanism of action of halogenated aryl hydrocarbons including PCBs, PCDDs and PCDFs have been extensively investigated [20-26]. Laboratory animal studies show that these compounds elicit a number of common toxic responses including: body weight loss, thymic atrophy and immunotoxicity, teratogenicity and reproductive problems, hepatotoxicity and porphyria, carcinogenicity, effects on the endocrine system and dermal toxicity. All of these responses are not evoked in any single animal species; however, body weight loss and thymic atrophy are the signs of toxicity most frequently observed in laboratory animals.

Most incidents involving environmental or accidental human exposure to the toxic PCDDs and PCDFs are associated with concomitant exposure to higher levels of PCBs and these are generally reflected in human tissue levels in which the PCBs/PCDDs, PCDFs ratios are $>1000:1$. For example in the Yusho and Yu Cheng poisoning in Japan and Taiwan respectively, several thousand individuals were poisoned with rice oil contaminated with PCBs and PCDFs [27,28]. The ratios of PCBs/PCDFs in the contaminated oil and the blood of exposed individuals were generally in the order of 184-667:1 and 1500-1890:1 respectively [27]. Since the interactive effects of concurrent exposure to halogenated aryl hydrocarbons is not well understood, this study reports teratogenicity of combined exposure to 2,3,7,8-TCDD and Aroclor 1254, a commercial PCB formulation.

MATERIALS AND METHODS

Chemicals

2,3,7,8-TCDD was prepared in our laboratory and the purity was $> 99\%$ as determined by gas-liquid and high-pressure liquid chromatographic analysis. Dexamethasone was purchased from Sigma Chemical Co., St. Louis, MO. Aroclor 1254 was a generous gift of Mr. B. Chittim, Wellington Environmental Consultants, Guelph, Ont., Canada.

Animal treatment

Mature male C57BL/6J mice and virgin female C57BL/6N mice (20-25 g) were purchased from Harlan Sprague-Dawley, Indianapolis, IN and maintained on food and water ad libitum at the Texas A&M University Laboratory Animal Resources

and Research facility. At the end of a 7-day acclimation period, females were mated with the males overnight. The following morning, females were checked for the presence of vaginal plugs. The presence of a vaginal plug was denoted as day 0 of pregnancy. Females with vaginal plugs were weighed and marked. Pregnancies were confirmed by measuring weight gain. The dams were treated by oral gavage with either corn oil (10 ml/kg), Aroclor 1254 (244 mg/kg), 2,3,7,8-TCDD (20 μ g/kg), Aroclor 1254 (244 mg/kg) followed by 2,3,7,8-TCDD (20 μ g/kg), dexamethasone (90 mg/kg), or Aroclor 1254 (244 mg/kg) followed by dexamethasone (90 mg/kg). All these compounds or mixtures were administered in corn oil (10 ml/kg) by oral gavage. Aroclor 1254 alone was administered on day 9 of pregnancy; corn oil and 2,3,7,8-TCDD were given on day 10 and dexamethasone on day 13. The animals receiving 2 chemicals were given Aroclor 1254 on the day prior to either 2,3,7,8-TCDD or dexamethasone, i.e. Aroclor 1254 on day 9 and 2,3,7,8-TCDD on day 10 or Aroclor 1254 on day 12 and dexamethasone on day 13 of pregnancy. Controls were either untreated or treated with corn oil (10 ml/kg). On day 17, the females were killed with CO₂ or by cervical dislocation. The weights of the dams and their respective liver weights were recorded. The uterine horns were exposed and the number of resorptions, dead and live fetuses were counted. Live fetuses were removed from the uterus, weighed and examined for gross abnormalities. The fetuses were fixed in Bouin's solution for at least 1 week and examined for internal soft tissue anomalies as previously described [29,30]. Statistical differences were determined using an analysis of variance procedure (ANOVA).

RESULTS AND DISCUSSION

Table I summarizes the effects of 2,3,7,8-TCDD, Aroclor 1254, 2,3,7,8-TCDD plus Aroclor 1254, dexamethasone and dexamethasone plus Aroclor 1254 on C57BL/6J females and their fetuses. No apparent signs of maternal toxicity were observed in any of the treatment groups. The percentages of fetuses with hydronephrosis per litter for the treatment groups were: untreated (1.3 ± 4.3); corn oil (5.7 ± 9.9), Aroclor 1254 (33.4 ± 18.0); 2,3,7,8-TCDD (87.8 ± 20.4); Aroclor 1254 + 2,3,7,8-TCDD (96.0 ± 11.0); dexamethasone (3.2 ± 7.3); and Aroclor 1254 + dexamethasone (3.7 ± 5.5). The dose of Aroclor 1254 (224 mg/kg) caused considerable hydronephrosis and, not surprisingly, did not antagonize the effects of 2,3,7,8-TCDD. Hydronephrosis is a more sensitive indicator of exposure to toxic halogenated aryl hydrocarbons [31,38,39] and at a dose level of 244 mg/kg, Aroclor 1254 acts as an agonist for this response. At a dose of 20 μ g/kg, 2,3,7,8-TCDD caused 61.8% fetal cleft palate per litter whereas Aroclor 1254 (244 mg/kg) did not cause any cleft palate in the litters. In contrast, hydronephrosis was observed in animals exposed to 2,3,7,8-TCDD or Aroclor 1254 (data not shown). Cotreatment of the mice with a teratogenic dose of 2,3,7,8-TCDD (20 μ g/kg) plus Aroclor 1254 (244 mg/kg) resulted in only 8.2% fetal cleft palate per litter which was significantly

TABLE I
EFFECTS OF AROCLOR 1254 PRETREATMENT ON 2,3,7,8-TCDD-INDUCED TERATOGENESIS

Treatment	No. of litters	Fetal body weight (g)	Maternal weight gain (g)	Maternal liver/body weight ($\times 100$)	Live fetuses per litter (%)	Resorptions of deaths per litter (%)	No. of litters with cleft palate	Fetuses with cleft palate per litter (%)
Untreated	11	0.79 $\pm$ 0.20	13.40 $\pm$ 3.68	5.60 $\pm$ 0.42	88.9 $\pm$ 11.5	11.1 $\pm$ 11.5	1	1.3 $\pm$ 4.3
Corn oil								
2,3,7,8-TCDD (10 ml/kg)	11	0.80 $\pm$ 0.20	15.58 $\pm$ 3.40	5.74 $\pm$ 0.33	91.8 $\pm$ 14.7	8.2 $\pm$ 14.7	0	0.0 $\pm$ 0.0
2,3,7,8-TCDD (20 μ g/kg)	10	0.94 ^a $\pm$ 0.21	15.70 $\pm$ 3.11	6.38 $\pm$ 0.67 ^a	87.8 $\pm$ 14.8	12.1 $\pm$ 14.8	10	61.8 $\pm$ 23.1 ^{a,b}
Aroclor 1254 (244 mg/kg)								
plus 2,3,7,8-TCDD (20 μ g/kg)	17	0.80 ^a $\pm$ 0.18	14.47 $\pm$ 3.69	9.25 $\pm$ 0.93 ^{a,c}	82.1 $\pm$ 16.4	17.9 $\pm$ 16.4	6	3.2 $\pm$ 1.5 ^a
Aroclor 1254 (244 mg/kg)								
Dexamethasone (90 mg/kg)	11	0.76 ^b $\pm$ 0.20	15.65 $\pm$ 2.15 ^a	7.41 $\pm$ 5.6	93.2 $\pm$ 5.6	6.8 $\pm$ 5.6	0	0.0 $\pm$ 0.0 ^a
Aroclor 1254 (244 mg/kg)	10	0.65 ^a $\pm$ 0.21	11.59 $\pm$ 1.30 ^a	6.02 $\pm$ 0.37 ^b	94.6 $\pm$ 7.7	5.4 $\pm$ 7.7	10	69.9 $\pm$ 18.2 ^{a,b}
plus dexamethasone (90 mg/kg)	9	0.64 ^a $\pm$ 0.21	12.50 $\pm$ 2.23 ^a	7.78 $\pm$ 9.3	88.8 $\pm$ 9.3	11.2 $\pm$ 9.3	9	85.8 $\pm$ 29.1 ^{a,b}

^aSignificantly different from corn oil-treated group at $P < 0.05$.

^{a,b}Values with such signs are significantly different from each other at $P < 0.05$.

decreased from the teratogenic effects observed with 2,3,7,8-TCDD alone.

These results contrast with previous studies on the interactive effects of individual PCBs and 2,3,7,8-TCDD as teratogens [31]. For example, the teratogenicity of 2,3,7,8-TCDD was unaffected by cotreatment with 2,2',4,4',5,5'-hexachlorobiphenyl whereas 2,3,3',4,4',5-hexachlorobiphenyl potentiated the teratogenicity of 2,3,7,8-TCDD. Previous reports have shown that 2,3,3',4,4',5-hexachlorobiphenyl evokes several typical 2,3,7,8-TCDD-like responses such as the induction of cytochrome *P*-450_c and related monooxygenases, body weight loss, thymic atrophy and immunotoxicity [32-36]. Moreover, this PCB congener binds with moderate affinity to the Ah receptor protein [37]. In contrast, 2,2',4,4',5,5'-hexachlorobiphenyl does not elicit Ah receptor-mediated effects in laboratory animals [20,33]. Subsequent studies on the interactive effects of 2,3,7,8-TCDD and 2,3,3',4,4',5-hexachlorobiphenyl as teratogens suggest that the interactive effects are additive [38] and recent work shows that the interactive effects of 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) as teratogens are also additive [39].

Although Aroclor 1254 is a complex mixture of PCB isomers and congeners, this mixture, like 2,3,3',4,4',5-hexachlorobiphenyl, also elicits a broad spectrum of Ah receptor-mediated responses including immunotoxicity, thymic atrophy, body weight loss, porphyria and the induction of cytochrome *P*-450_c and related monooxygenases and exhibits moderate affinity for the Ah receptor protein [20,32-37]. In fact, both Aroclor 1254 and 2,3,3',4,4',5-hexachlorobiphenyl are weak Ah receptor agonists compared to 2,3,7,8-TCDD and their competitive binding affinities for the hepatic Ah receptor are comparable [37]. One major difference between 2,3,3',4,4',5-hexachlorobiphenyl and Aroclor 1254 is the relative potency of the former compound. For example, the *in vivo* ED₅₀ values for body weight loss, thymic atrophy and hepatic microsomal aryl hydrocarbon hydroxylase in the rat for 2,3,3',4,4',5'-hexachlorobiphenyl are 180, 180 and 7 $\mu\text{mol/kg}$ [36]; the corresponding values for Aroclor 1254 are at least 10 times higher [40]. Moreover, Aroclor 1254 exhibits a competitive Ah receptor binding affinity only 500 times lower than 2,3,7,8-TCDD whereas the toxicity and enzyme induction activity of the latter compound is at least 10⁵ times greater in the rat [41]. Although a direct comparison of the teratogenic potency of Aroclor 1254 and 2,3,7,8-TCDD has not been made, the ED₅₀ for teratogenicity (cleft palate) in this study was < 20 $\mu\text{g/kg}$ whereas the ED₅₀ for Kanechlor 500 in the ddY strain of mice was >2000 $\mu\text{mol/kg}$ [42]. We therefore propose that Aroclor 1254 may act as a partial 2,3,7,8-TCDD antagonist by competing for the Ah receptor and thus lowering the cellular levels of the Ah receptor available for interaction with the potent agonist, 2,3,7,8-TCDD. Evidence which supports this hypothesis include the following: (a) cotreatment of C57BL/6J mice with Aroclor 1254 and 2,3,7,8-TCDD at Aroclor 1254/2,3,7,8-TCDD ratios >1000:1 showed that Aroclor 1254 can significantly antagonize the induction of 2 Ah receptor-mediated responses, namely the induction of aryl hydrocarbon hydroxylase (AHH) and the plaque-forming cell response to sheep

erythrocytes [43]; (b) Aroclor 1254 also antagonizes the induction of AHH by 2,3,7,8-TCDD in rat hepatoma H-4-II E cells in culture [43]; (c) double-reciprocal plot analysis of the Ah receptor-2,3,7,8-TCDD binding isotherms in the presence of different concentrations of Aroclor 1254 gave data which are consistent with competitive inhibition of 2,3,7,8-TCDD-receptor binding by Aroclor 1254 [43]; (d) Aroclor 1254 does not act as a non-specific teratogen antagonist since Aroclor 1254 does not antagonize the teratogenicity of dexamethasone, a synthetic glucocorticoid hormone analog which acts through initial binding with the glucocorticoid receptor (see Table I).

These results further extend the spectrum of 2,3,7,8-TCDD-mediated responses which can be antagonized by Aroclor 1254. Moreover since human environmental exposure to the toxic PCDD and PCDF congeners occurs in concert with simultaneous exposure and bioaccumulation of PCBs [1-4,9-16], it is possible that the non-toxic environmental PCB levels may afford some measure of protection from these toxins since the environmental ratios of PCB/toxic PCDDs + PCDFs [7-9,12-15] are comparable to ratios in which Aroclor 1254 exhibits antagonistic activity (this paper; [43]). Current studies in our laboratory are focused on further delineating the mechanism of the interactive effects of commercial PCBs and individual PCB congeners with the toxic 2,3,7,8-substituted PCDDs and PCDFs.

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