COMPARISON OF CLEFT PALATE FREQUENCY INDUCED BY 2,3,7,8-TETRABROMODIBENZO-P-DIOXIN AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN MICE

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ABSTRACT

We studied the potency of 2,3,7,8-tetra*bromo*dibenzo-*p*-dioxin (TBrDD) to induce cleft palates in mice. These data were compared with the results obtained with 2,3,7,8-tetra*chloro*dibenzo-*p*-dioxin (TCDD) under identical experimental conditions.

NMRI mice were treated s.c. with single injections of TBrDD or TCDD (5 - 90 μg/kg body wt) on day 9 of pregnancy, and maternal and fetal toxicity were assessed on day 18.

Analysing the dose-response for cleft palate induction with the probit model TBrDD was found to exhibit a potency of 0.6 on a molar basis when compared with that of TCDD.

KEY WORDS

2,3,7,8-Tetra*bromo*dibenzo-*p*-dioxin; 2,3,7,8-Tetra*chloro*dibenzo-*p*-dioxin; Cleft palate; NMRI mice

ABBREVIATIONS

TBrDD = 2,3,7,8-Tetra*bromo*dibenzo-*p*-dioxin; TCDD = 2,3,7,8-Tetra*chloro*dibenzo-*p*-dioxin

INTRODUCTION

2,3,7,8-Tetra*chloro*dibenzo-*p*-dioxin (TCDD), an environmental pollutant of high toxicity, also exhibits an embryotoxic potency at comparatively high doses. In mice the most pronounced effect is the induction of cleft palates (Courtney and Moore, 1971; Neubert and Dillmann, 1972).
Halogenated aromatics containing one or more bromine atoms have been produced as commercial chemicals. Model laboratory studies have demonstrated that pyrolysis of brominated flame retardants results in the formation of polybrominated dibenzo-*p*-dioxins (PBrDDs) and dibenzofurans (PBrDFs), including congeners of high toxicity (Thoma et al., 1986). Thus, PBrDDs/PBrDFs may be of significance as environmental pollutants. Little comparable information, however, is available on the toxic potential of these substances, whereas an impressive amount of data exists on the toxic effects of TCDD.
We therefore studied the potency of TBrDD to induce cleft palates in mice and compared the potency with that of TCDD.

MATERIALS AND METHODS

Animals
The studies were performed on random-bred albino mice (NMRI) kept under conventional conditions at a constant temperature (21 ± 1°C) and relative humidity (50 ± 5%) and at a constant light-dark cycle (dark period from 8:00 p.m. to 9:00 a.m.). The animals were fed Altromin^{R} 1324 and tap water ad libitum. Mice weighing 29-35 g were mated for 2 h (7:00 a.m. to 9:00 a.m.). When vaginal plugs were detected the 24 h period following the mating period was designated day 0 of pregnancy.

Chemicals
^{14}C-TCDD was purchased from Cambridge Isotope Lab., Mass., USA. It had an indicated specific activity of 33 mCi (1.22×10^{9} Bq)/mmole. TBrDD was purchased from Promochem, Wesel, FRG (Lot No. MLB 13648-1), and had a chemical purity of 98% (according to the manufacturer). The substances were dissolved in a mixture of toluene/DMSO (1+2, v/v) and kept in the dark.

Teratological experiments
TBrDD and TCDD (5 - 90 μg/kg body wt) were administered on day 9 of pregnancy by single subcutaneous injection. An injection volume was held constant at 40 μl per 40 g body wt. Controls were treated with a mixture of toluene/DMSO (1+2, v/v) at the same time. Maternal liver weight and the weight of viable fetuses were evaluated on day 18 of pregnancy, and the number of implantation sites, resorptions, late deaths, and fetuses were recorded. Viable fetuses were evaluated for externally visible malformations under a stereo-microscope, fixed in 5% formol for 5 days and evaluated for cleft palates.

Statistical evaluations
Statistical significance was evaluated with the Chi^2-test, Student's *t*-test or the Mann-Whitney test. Dose-response curves were calculated using a programm for probit analysis yielding the regression curve and the 95% confidence intervals. This analysis was based on the overall percentage of affected fetuses in each dose group.

RESULTS AND DISCUSSION

Both TCDD and TBrDD at the doses given induced only marginal toxic symptoms in the treated animals, and there was no significant effect on maternal weight gain. A significant increase in maternal liver to body weight ratios was observed when compared to the controls (Table 1). This finding is a typical effect of comparatively high doses of polyhalogenated dioxins and furans (Goldstein, 1980; Poland and Knutson, 1982).

Despite some maternal effects, administration of TBrDD or TCDD on day 9 of pregnancy did not affect the number of viable fetuses per litter or fetal mortality.
With respect to the induction of cleft palates, TBrDD as well as TCDD produced significant dose-related increases in the incidence of cleft palates, as well as in the number of litters with fetuses with cleft palate, and in the total number of viable fetuses per dose group (Table 2).

Table 1. Body and liver weights in pregnant mice after injection of TBrDD and TCDD

Congener	Dose (μg/kg)	Maternal weight gain (g)[a] (M ± S.D.)	Maternal liver/body weight x 100 (M ± S.D.)
TBrDD	5	1.5 ± 1.3	7.6 ± 0.5**
	10	2.4 ± 1.0*	8.0 ± 0.5**
	30	1.4 ± 1.8	8.0 ± 0.4**
	50	1.1 ± 1.5	8.2 ± 0.5**
	90	1.0 ± 1.3	7.7 ± 0.5
TCDD	5	1.4 ± 2.3	7.0 ± 0.5**
	15	1.6 ± 1.0*	7.6 ± 0.5**
	30	2.0 ± 1.4	8.0 ± 0.3**
	45	1.2 ± 1.6	8.0 ± 0.5**
	90	0.7 ± 1.0	8.3 ± 0.5
Control	vehicle	1.0 ± 0.9	6.9 ± 0.3

a : Maternal weight gain was defined as the difference between the weight of dams on day 18 (without uterus) minus the weight on day 9, the day of dosing.
* : $p < 0.05$;
** : $p < 0.01$

Table 2. Comparison of the teratogenic potency of TBrDD and TCDD in mice

Congener	Dose (μg/kg)	Fetal weight (g) (M ± S.D.)	Cleft palate/fetuses (%)	ED_{50} μg/kg	ED_{50} μmole/kg
TBrDD	5	1.16 ± 0.07	1/90 = 1.1		
	10	1.18 ± 0.07	19/157 = 12.1		
	30	1.16 ± 0.08	39/198 = 19.7	61.7	0.123
	50	1.18 ± 0.09	103/185 = 55.7	± 4.8	
	90	1.23 ± 0.07	118/200 = 59.0		
TCDD	5	1.26 ± 0.09*	8/148 = 5.4		
	15	1.15 ± 0.12	41/146 = 28.1		
	30	1.12 ± 0.08	86/154 = 55.8	24.0	0.075
	45	1.18 ± 0.09	156/202 = 77.2	± 1.3	
	90	1.20 ± 0.19	53/62 = 85.5		
Controls	vehicle	1.17 ± 0.13	2/443 = 0.5		

From these data the regression equations for TBrDD and TCDD were calculated. The slope of the dose-response curve of both substances were: 1.6 ± 0.1 for TBrDD and 2.2 ± 0.2 for TCDD, respectively. ED_{50} (± S.D.) values for cleft palate formation were 61.7 ± 4.8 μg/kg (= 0.123 μmole/kg) for TBrDD and 24.0 ± 1.3 μg/kg (= 0.075 μmole/kg) for TCDD.

Thus, we conclude that under the present experimental conditions TBrDD is a powerful inducer of cleft palates in NMRI mice with a potency of about 60% of that of TCDD when evaluated on a molar basis.

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