FETAL AND INFANT BODY BURDEN OF 2, 3, 7, 8-TETRACHLORODIBENZO-*p*-DIOXIN CAUSING A SHORT ANOGENITAL DISTANCE AND IMMUNOTOXICITY IN MALE HOLTZMAN RATS

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Introduction

Maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes a variety of toxic responses in their fetus and offspring. The effects include decreased birth weight, irreversible changes in the reproductive systems[1-5], feminized sexual behavior[6], and change of thymocyte subpopulation[7]. Recently, we also observed suppression of development of reproductive organs and decrease of splenocyte number in male pups following *in utero* and lactational TCDD exposure[8, 9]. Bjerke and Peterson compared the effects of *in utero* versus lactational TCDD on male reproductive function[10], and concluded that male reproductive effects was caused predominantly by low level TCDD exposure via *in utero* route. Furthermore, Gray *et al.* and our group showed that critical period was gestation day (GD) 15 on the development of male reproductive organs in rat[11]. Gehrs *et al.* compared the severity of the immunotoxic effects between *in utero* and lactational TCDD exposure[12]. The order of severity was via lactational with *in utero*, lactational alone, *in utero* alone. Therefore, body burden of TCDD at the end of lactation is considerably important to estimate the immunotoxic effects. The objective of the current study is to determine the fetal and infant body burden at the critical period for male reproductive and immune organs by using high-resolution GC/MS analysis.

Materials and Methods

Animals and treatments

The following administration and necropsies were all performed in the hazardous chemical regulation area at our institute. Pregnant Holtzman rats (5 per group) were given a single oral dose of 0, 50, 200 or 800 ng TCDD/kg body weight on GD 15. Dams were sacrificed under diethylether-anesthesia on GD 16 and postnatal day (PND) 21, and blood, adipose tissue and fetuses were collected. Tissue and blood specimens were collected from 5 infant rats (one per litter) of each group on PND 21, 49 and 120. The blood was centrifuged at 900 x *g* for 15 min and the plasma was stored at -80°C until GC/MS analyses.

TCDD analysis

The content of TCDD was determined according to the methods described previously with a minor modification[13]. Briefly, tissue specimens (0.1-1g) were spiked with ^{13}C-2,3,7,8-TCDD as internal standard, and digested in 2 M potassium hydroxide solution. The digested material was extracted with n-hexane, and the extract was washed with concentrated sulfuric acid. The n-hexane layer was concentrated and sequentially subjected to silica gel, alumina and active carbon impregnated-silica gel column chromatographies. The GC/MS analysis was performed in the selected ion mode with a JMS 700 high-performance double-focusing mass spectrometer (JEOL, Japan) coupled to an HP 6890 gas chromatograph (Hewlett Packard, USA) with CP-SIL 8CB/MS column (Varian, USA).

Results and Discussion

Effects of maternal TCDD exposure on male reproductive organs

The anogenital distance and ventral prostate weight of male rats sacrificed on PND 120 showed a significant decrease in the groups receiving doses greater than 50 and 200 ng TCDD/kg, respectively. TCDD concentration was determined in fetus on GD16 as critical period, and in adipose tissue on PND120. There was a dose-dependent increase in the fetal body burden and TCDD concentration in adipose following maternal exposure to 50, 200 or 800 ng TCDD/kg. The lowest observed maternal dose that suppressed the development of male pup reproductive organ was 50 ng TCDD/kg, which resulted in fetal body burden of 7.9 ng/kg on GD16 (Table 1).

Effects of maternal TCDD exposure on male immune organs

Splenocyte number was decreased by maternal exposure to 12.5 - 800 ng TCDD/kg in a dose-dependent manner on PND 49, and significant decrease was observed in the group receiving 800 ng TCDD/kg. Immunotoxic effects in pups were caused by both *in utero* and lactational TCDD exposure, therefore we determined TCDD concentration in whole pup at weaning, and immune organs on PND 49. The maternal TCDD dose of 800 ng/kg resulted in a body burden of 285 ng/kg in pup on PND 21(Table 1). The thymus and spleen maternally exposed to 800 ng TCDD/kg contained 28.0 and 22.4 pg TCDD/g-tissue on PND 49, respectively.

Table 1. Effects of maternal TCDD exposure and body burden in the rat

Biological effects	Exposure (LOEL or LOAEL)		Body burden (ng TCDD/kg)		
anogenital distance [8]	50		GD16 fetus	7.9	
ventral prostate weight [8]	200		GD16 fetus	33	
splenocyte number [9]	800		PND21 pup	285	
anogenital distance [8]		50	maternal		43 *
ventral prostate weight [8]	200		maternal	172 *	
splenocyte number [9]	800		maternal	688 *	
sperm count in testis [3]		64	maternal	55 *	
sperm count in epididymis [2]	200		maternal	172 *	
birth weight [1]		400	maternal	344 *	
immunotoxicity [7]	1000		maternal	860 *	

*: Calculated from the exposure condition of original report. (assumes a gastrointestinal absorption of 86 % peroral treatment with corn oil)

For risk assessment of the maternal TCDD exposure on pups, TCDD concentration in target organs of fetus/pup at the critical period is more meaningful than maternal dose or body burden. In the present study, fetal body burden on GD 16 was approximately a quarter of calculated maternal body burden. Decrease of anogenital distance of male pup was caused by fetal body burden of 7.9 ng TCDD/kg or more. This fetal body burden is comparable to the body burden of 5 ng TEQ/kg in adult humans with no known excessive exposure to dioxins and related compounds. This result indicates that margin of exposure (MOE) to TCDD could be 5-10 times lower than the MOE level so far reported. Although no obvious evidence on the occurrence of signs and symptoms due to dioxin and related compounds has been documented, the probabilistic risk of occurrence of some subtle change is thought to be non-negligible as low as exposure to dioxin and related compounds remain at the current level.

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