In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys

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Introduction

The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.* reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring[1]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI was established. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of this calculation is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999.

In rodents, teeth are known to be targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[2,3]. In humans also tooth abnormalities were reported among populations exposed to dioxins[4]. In our monkey experiment, some young were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate young exposed to TCDD *in utero* and lactationally. By macroscopic observation we found some tooth abnormalities among died young exposed to TCDD[5]. This prompted us to examine surviving young by radiography. This is an interim report of our findings in these young.

Methods and Materials

Animals: Adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China) were used. Details of breeding conditions were given elsewhere[6]. Female monkeys were allowed to cohabit with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period was disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups each consisting of approximately 20 animals and allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PD0).

Administration of TCDD: TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy and lactation until day 90 after birth.

Measurement of TCDD in maternal serum: Approximately 20 ml of blood was taken from the femoral vein of the dams on day 80 of pregnancy, and centrifuged. The obtained serum was subjected to high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) by the method of Patterson *et al.*[7]

Observation of teeth of the young: Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Surviving young were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

Pregnancy outcome and postnatal development of the young: Table 1 summarizes the pregnancy outcome and postnatal mortality of the young. Abortions, stillborns, and postnatal deaths occurred fairly frequently even in the control group. To increase the number of surviving young in the 300 ng/kg, we added 9 dams to the group approximately 2 years after the initiation of the experiment. However, only two young survived more than a year due to a high incidence of abortions. No significant differences were noted in the gestation length and birth weight among the control and TCDD-treated groups, indicating the body burden of TCDD at 300 ng/kg did not affected general growth of the young.

Table 1: Pregnancy outcome and postnatal mortality of rhesus monkeys exposed to TCDD.

Group	No. of dams	No. of abortions	No. of stillborns	No. of live borns	No. of postnatal deaths	Gestation length (days)	Birth weight (g)
Control	23	2	3	18	5	161.8±7.8	426.1±58.6
30 ng/kg	20	0	5	15	3	163.8±5.9	426.8±56.9
300 ng/kg	20	2	2	16	8	164.9±9.7	408.6±63.7
300 ng/kg[1)]	9	5	1	3	1	165.0±3.0	466.0±87.1

1) Newly added group

Tooth abnormalities in the young: The incidence of tooth abnormalities in the young was shown in Table 2. Tooth abnormalities in the stillborn and postnatally died young were described previously[5]. No abnormalities were detected in the control and 30 ng/kg groups, whereas more than half of the young in the 300 ng/kg had tooth abnormalities as listed in Table 3. The upper permanent lateral incisors were most frequently affected. In contrast, among the deciduous teeth, the central incisors seemed to be most sensitive targets of developmental toxicity of TCDD. The permanent premolars were also affected frequently, while the canine and the first molar were resistant to the adverse effect of TCDD. Probably these larger teeth have become resistant to odontotoxic chemicals during the course of evolution.

Table 2: Incidence of tooth abnormalities among F1 exposed to TCDD.

Group	Stillborns and postnatally died young			Surviving young		
	No. of specimens	No. of specimens with tooth abnormalities (%)		No. of young	No. of young with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	12	0	(0)
300 ng/kg	8	3	(38)	8	6	(75)
300 ng/kg[1)]	2	0	(0)	2	1	(50)

1) Newly added group

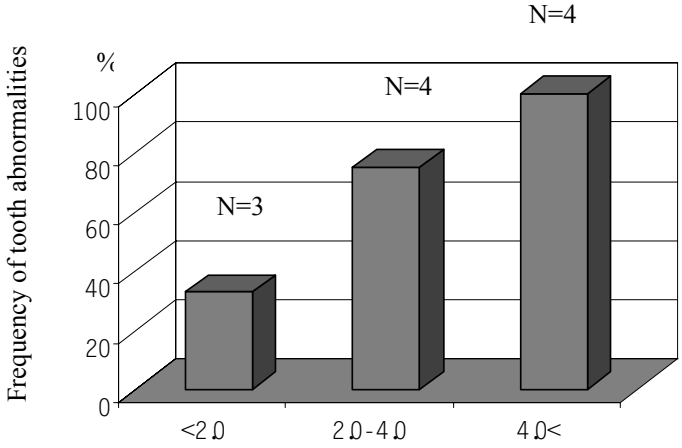
Relationship between maternal serum TCDD concentration and occurrence of tooth abnormalities: In the control maternal serum, the TCDD levels were below the detection limit. In the 30 ng/kg group, the levels were fairly constant, ranging from 0.19 to 0.21 pg/g wet weight. In contrast, the levels varied largely in the 300 ng/kg group, ranging from 1.1 to 8.9 pg/g wet weight. The average of those without tooth abnormalities in their young was 1.4 ± 0.6 pg/g wet weight, whereas that with tooth abnormalities was 4.3 ± 2.4 pg/g wet weight. The concentration-response relationship is shown in Fig. 1.

Table 3: Tooth abnormalities detected in the young exposed to TCDD at 300 ng/kg.

Young	Sex	Age (days) [2)]	Abnormal Findings
31	♀	1430	542|24 missing |5 conical
39	♂	1410	542|245 missing
42	♀	1415	5 |5 missing |4 conical
44	♂	1415	54 |45 missing |5 conical
60	♂	1388	542|245 5|5 missing
66	♂	1338	52|2 1|1 missing 54 |45 malaligned |45 conical
106[1)]	♀	688	A |A 4 |24 missing

1) Newly added group
2) Age at X-ray examination

Figure 1: Maternal serum concentration of TCDD and the incidence of tooth abnormalities.



Maternal serum concentration of TCDD (pg/g wet weight)
N = number of specimens in each concentration category

Validity of the current TDI: The above results indicate that the LOAEL body burden for induction of tooth abnormalities in the rhesus monkey is at a certain level between 30 ng/kg and 300 ng/kg, probably not much different from the LOAEL body burden for rodents, 86 ng/kg. Hence it is reasonable to conclude that the current TDI of dioxins in Japan needs no immediate modification.

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