EFFCT OF *IN UTERO* AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ON TOOTH DEVELOPMENT IN RHESES 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[1]. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.*[2] 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. 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 rodents, teeth are known to be targets of developmental toxicity of dioxin. In utero and lactational TCDD exposure affects rat incisor and molar developmet[3, 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. This is a preliminary report of our findings in these young.

Materials and Methods

Animals and treatment. Adult rhesus monkeys were mated, and females with confirmation of pregnancy by ultrasonography were given TCDD subcutaneously on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. Controls received the vehicle. Approximately 20 dams were allotted to each dose group. The lower dose level was set at about one third of the LOAEL body burden in rodents, and the higher one at about three times of the LOAEL. For maintenance of a certain body burden, 5% of the initial dose was given to dams every 30 days during pregnancy and lactation until day 90 after birth. After weaning of the first born young (F1a) the dams were remated to obtain the second born young (F1b), and TCDD was given to maintain the body burden.

Table 1. Observed specimens and their findings

Group	Specimen No*.	Status	Age**	Findings***
Control	10a	stillborn	GD146	no abnormalities detected
	13a	stillborn	GD156	no abnormalities detected
	49a	stillborn	GD147	no abnormalities detected
	58a	postnatal death	PND98	no abnormalities detected
30 ng/kg	20a	stillborn	GD163	no abnormalities detected
	28a	stillborn	GD158	no abnormalities detected
	29a	stillborn	GD157	no abnormalities detected
	50a	stillborn	GD172	no abnormalities detected
	16b	stillborn	GD148	no abnormalities detected
	17b	stillborn	GD150	no abnormalities detected
	53b	stillborn	GD160	no abnormalities detected
	59b	stillborn	GD159	no abnormalities detected
300 ng/kg	34a	abortion	GD128	no abnormalities detected
	37a	stillborn	GD164	Up AAD Pr, Dy; Up LeB Mi
	40a	postnatal death	PND26	Up AA Mi; Up Le D Pr
	43a	stillborn	GD176	no abnormalities detected
	57a	postnatal death	PND1	Up AA Pr Mo; Up BB Lo AA Pr
	60b	postnatal death	PND25	no abnormalities detected
	66b	stillborn	GD143	no abnormalities detected
	103a	stillborn	GD173	no abnormalities detected

*Maternal ID number. 'a' and 'b' indicate first and second born young, respectively.
**GD: gestation day; PND: postnatal day.
***Up: upper; Lo: lower; Le: left; A: central incisor; B: lateral incisor; D: first molar; Pr: precociously erupting: Dy: dysgenetic; Mi: missing; Mo: mobile.

Macroscopic observation. Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Macroscopic observation was made under a dissecting microscope (Olympus SZX12). Photographs were taken by a digital camera (Olympus C-4040).

Radiographic observation. Conventional intraoral radiographs were taken by a portable X-ray apparatus (Asahi Roentgen KX-60) with a CCD sensor (Gendex Visualix). CT examination was done by a high resolution dento-maxillofacial cone beam X-ray CT system (Hitachi CB MercuRay) and three dimensional images were reconstructed by a computer with a software (ExaVison LITE).

Results and Discussion

Number of observed specimens and their findings are listed in Table 1. No abnormalities were detected in the control and 30 ng/kg groups. In the 300 ng/kg group, three of the eight observed specimens had abnormally developed teeth. Figures 1-3 illustrate abnormalities in a case. These findings suggest that teeth are sensitive targets of developmental toxicity of dioxin in primates.

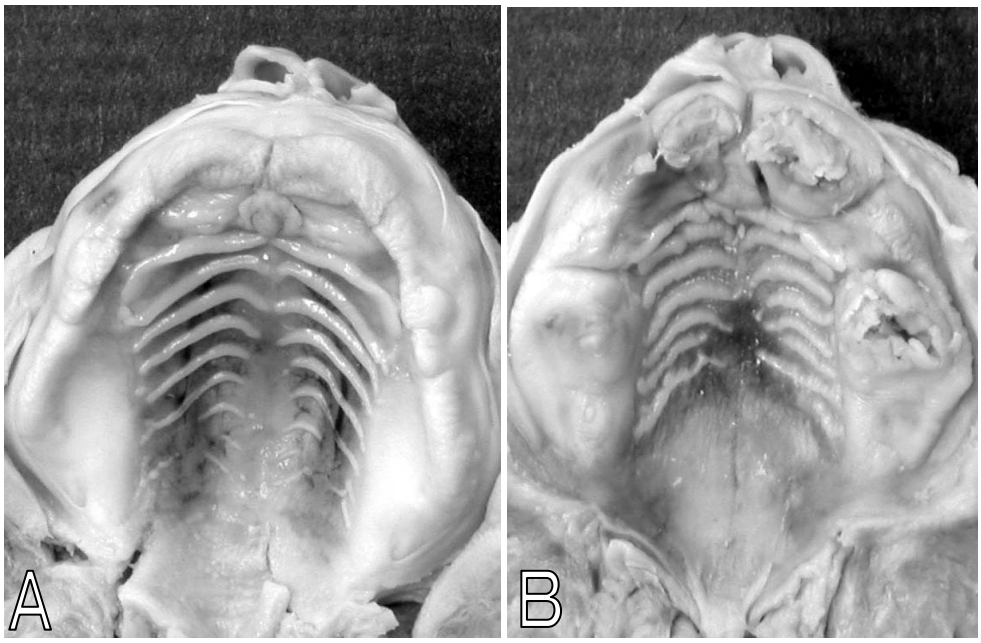

Fig.1. A: The upper jaw viewed from the oral cavity of a young stillborn on day 156 of gestation in the control group (13a). No teeth have erupted. B: The upper jaw of a young stillborn on day 164 of gestation in the 300 ng/kg group (37a). The central incisors and the left first molar have erupted precociously, and are dysgenetic.

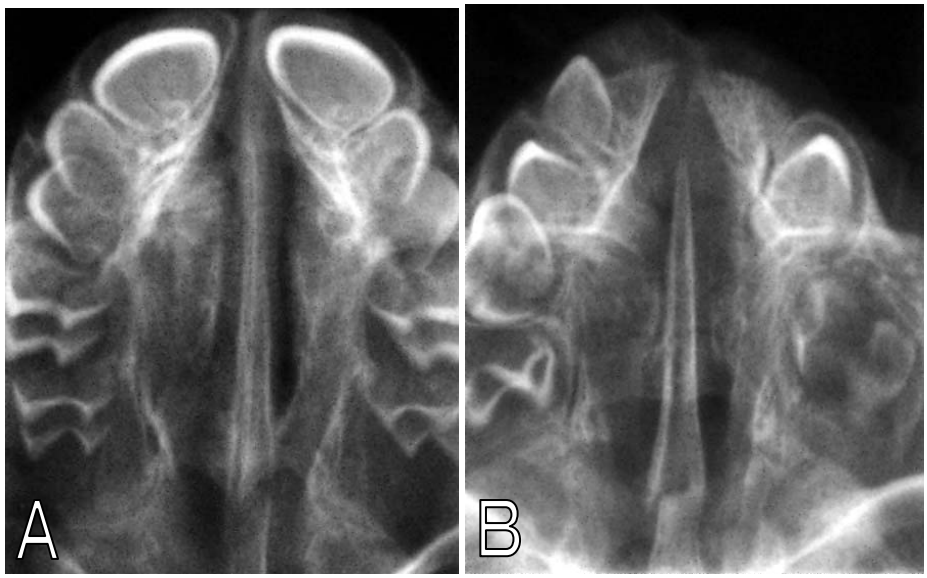

Fig.2. A, B: X-ray view of the upper jaw shown in Fig. 1A and B, respectively. In B, the precociously erupted central incisors and left first molar are not well calcified, the left lateral incisor is missing, and the left second molar is dislocated anteriorly.

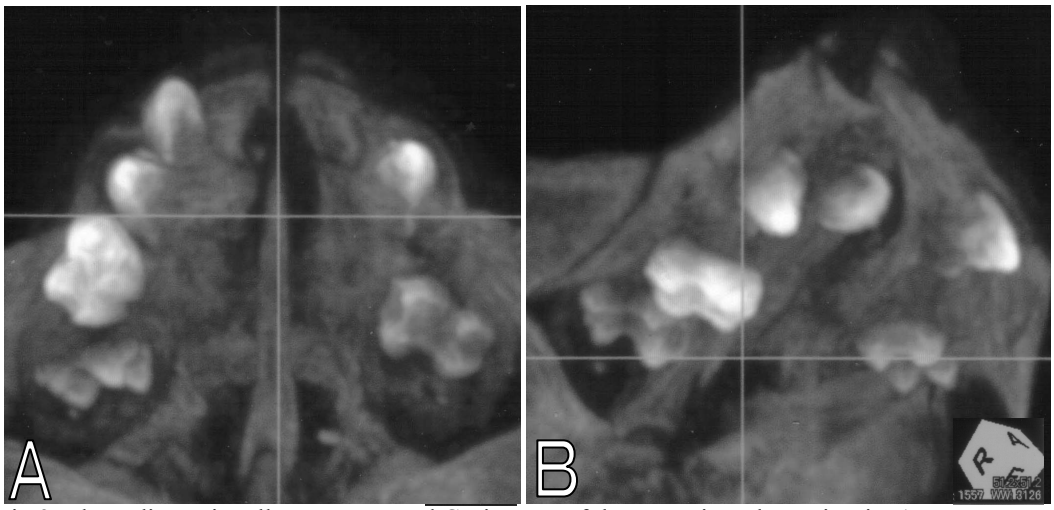

Fig.3. Three dimensionally reconstructed CT images of the upper jaw shown in Fig. 1B.
A: Inferior view. B: Right inferior oblique view. Note poor calcification of both central and left lateral incisors, and the left first molar.

In humans, it has been reported that demarcated hypomineralizations of developing teeth are a biological indicator of an early dioxin exposure in children[5]. For further evaluation of tooth development in our monkey population, observation of surviving young is in progress.

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