Western blot analysis of liver tissues: Control monkey has positive band at 110kD which corresponds to molecular weight of AhR. TCDD-injected ones have no positive band at the same position. On the other hand, positive bands as Arntl were observed both control and TCDD groups.

Discussion

These histopathological findings found in the liver of monkeys which were administrated with TCDD suggest sinusoidal endothelical cell injury and impairment in intrasinusoidal microcirculation because infarction, focal fatty change, and microthrombi-formation, that are rare events in the liver[8], are considerated to be closely associated with intrahepatic circularatoy impairment. As the hepatic parenchyma is protected against ischemia by its double blood supply, hepatic infarction is an uncommon lesion and usually accompanied by impairment in hepatic arterial and portal blood supply. Focal fatty change[8] is also unusually identified lesions which are first described in human in 1980 and becomes increasingly recognized by imaging techniques. It is surprising that these rare lesions were thus frequently identified in the liver of TCDD-treated rhesus monkeys. It is possible that small hepatocyte hypercellularity and alpha-SMA-positive satellite cell hyperplasia or transformation result from intralobular circulatory disturbance with local hypoxia and ischemic injury. Increased number of alpha-SMA-positive cells may suggest perisinusoidal fibrosis in the liver. There is no previous report describing these finding in the liver after injection with TCDD into the animal models although it has been reported that TCDD induced endothelical cell injury. It remains unclear whether or how TCDD induced intrasinusoidal endothelical cell injury. Further studies are necessary to explore the mechanism.

Acknowledgment

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Figure 1. Hemorrhagic infarction of the liver detected in rhesus monkey injected with TCDD.

Table 1. Histopathological findings in the liver of TCDD- administrated rhesus monkeys.

	Positive /total number examined in monkeys injected with TCDD		
	Control	30ng/kg	300ng/kg
Focal fatty change	0/3	2/4	2/3
Fatty change	0/3	1/4	1/3
Infarction	0/3	1/4	1/3
Hemorrhage	0/3	1/4	1/3
Microthrombi	0/3	2/4	2/3
Sinusoidal ectasia	0/3	3/4	2/3
Small cell hypercellularity	0/3	2/4	3/3
Alpha-smooth muscle actin-Positive cell hyperplasia	0/3	3/4	3/3

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

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

Abstract

We thought to validate the current tolerable daily intake (TDI) value for dioxin (4 pg/kg) in Japan. Pregnant rhesus monkeys received an initial dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 0, 30, or 300 ng/kg subcutaneously) on day 20 of gestation; the dams received additional injection of 5% of the initial dose every 30 days until day 90 after delivery. The teeth of stillborn, postnatally dead, and surviving offspring (now approximately 4 years old) were evaluated. None of the offspring in the 0- and 30-ng/kg groups (n=17 and 15, respectively) had tooth abnormalities, whereas 10 of 16 in the 300-ng/kg had them. These findings suggest the lowest-observed-adverse-effect-level (LOAEL) for TCDD in the rhesus monkey is between 30 and 300 ng/kg, and probably is close to that for rodents (86 ng/kg), on which the current TDI was based. It is reasonable to conclude that the current TDI needs no immediate modification.

Keywords: Dioxin; TCDD; Tooth; Rhesus monkey; Primate; TDI; Developmental toxicity; LOAEL

1. Introduction

Dioxins are ubiquitous environmental pollutants. Although contamination levels are decreasing [1], the adverse effects of dioxins, especially their reproductive and developmental toxicities, still attract much public concern, and regulatory agencies worldwide are seeking to define a reasonable permissible intake level. In Japan, the current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg toxic equivalent (TEQ)/kg/day [2]. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. 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 [3]. 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 of 4 pg/kg was established. However, great differences between the biological half-life of TCDD in humans and rodents have called into question the validity of this calculation. To obtain a more reliable LOAEL for dioxins, in 1999 we initiated a long-term developmental toxicity study in rhesus monkeys.

In rodents, the teeth are known targets of the developmental toxicity of dioxin; *in utero* and lactational TCDD exposure affects incisor and molar development in rats [4]. Tooth abnormalities also occurred among human populations accidentally exposed to dioxins [5] or polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDF) [6,7]. During our monkey experiment, some offspring were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate offspring exposed to TCDD *in utero* and while nursing. Macroscopic observation revealed tooth abnormalities in the offspring from mothers exposed to a relatively high dose of TCDD (300 ng/kg on day 20 of gestatoion and 15 ng/kg every 30 days during pregnancy) [8]. This finding prompted us to examine surviving offspring radiographically, and we found that tooth abnormalities occurred at a high frequency in the high-dose group. These offspring are still alive and growing, and various studies are in progress. This report describes the dental findings obtained as of April, 2004.

2. Materials and methods

2.1. Animals

Colony bred adult female rhesus monkeys (age, 3-10 years; weight, 4-7 kg) were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China). Details of breeding conditions are given elsewhere [9]. Briefly, the animals were housed in stainless-steel cages (68 cm × 70 cm × 77 cm), and received approximately 144 g of solid diet (Harlan Tekland, Harlan Sprague Dawley Inc., Indianapolis, IN) daily. The rooms were maintained at 26±2℃ and 50±10% relative humidity and on a 12-h light cycle (lights on, 0600-1800 h). Female monkeys were allowed to cohabit with males on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period (day13 of the menstrual cycle) was designated as day 0 of gestation (GD 0). On GD 18 or 19, pregnancy was confirmed by ultrasonography (SSD-2000, Aloka Co., Ltd, Tokyo, Japan) of animals anesthetized by an intramuscular injection of 5% ketamine hydrochloride (5-10 mg/kg, Sigma-Aldrich Corporation, St. Louis, MO). Pregnant monkeys were divided into three groups, each consisting of approximately 20 animals. During gestation, all dams were observed for general condition at least once daily and they were weighed once every 20 days.

The dams were allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PND 0). Delivered offspring were examined macroscopically, and allowed to cohabit with their mothers for approximately 1 year. The offspring were weighed once every 10 days until PND 90, once every 20 days until PND 150, and once every 30 days thereafter. The animals were reared in the monkey facility of Shin Nippon Biomedical Laboratories, Ltd. (SNBL, Kagoshima, Japan) and were treated humanely according to the guidelines of animal experiments for SNBL. Animal excreta and carcasses were handled with extreme care, and all waste was burned in an incinerator equipped with an afterburner held at >800℃.

2.2. Chemicals and administration

TCDD (lot number 110899, purity >98% as determined by gas chromatography, Wellington Laboratories Inc., Guelph, Ontario, Canada) was dissolved in a mixture of toluene/dimethylsulfoxide (DMSO; 1:2, v/v) at a concentration of 300 ng/ml. The solution was prepared by Kanto Kagaku Co., Ltd. (Tokyo, Japan) and final concentrations were confirmed by gas chromatography. Confirmed pregnant female monkeys received TCDD subcutaneously into the

back region on GD 20 at an initial dose of 30 or 300 ng/kg. This route was selected to avoid uncertainty of absorption by oral administration. The dosing volume was 0.1 ml/kg for the lower-dose group and 1 ml/kg for the higher-dose group. Controls received the vehicle in a volume of 1 ml/kg. To maintain the desired body burden, dams received 5% of the initial dose (i.e. 1.5 or 15 ng/kg) every 30 days during pregnancy and lactation until PND 90. For the maintenance dosing, a TCDD solution at a concentration of 30 ng/ml was prepared, and animals in the lower-dose group received 0.05 ml/kg in each injection whereas those in the higher-dose group received 0.5 ml/kg. The total dose administered to the higher-dose group was 405 (300 + 15 × 7 for dams with gestation length less than 170 days) ng/kg, or 420 (300 + 15 × 8 for dams with gestation length 170 days or more) ng/kg, and that to the lower-dose group was 40.5 or 42 ng/kg. The lower dose level was set at about one third of the LOAEL body burden in rodents (86 ng/kg) and the higher one at about three times the LOAEL. The maintenance dosing schedule was set according to the assumption that the biological half-life of TCDD in rhesus monkeys is approximately 1 year [10].

2.3. Macroscopic observation

Stillborn fetuses and offspring that died by PND 100 were necropsied, and the upper and lower jaws were dissected for detailed observation. Macroscopic observation was made under a dissecting microscope (SZX12, Olympus Corporation, Tokyo, Japan). Photographs were taken using a digital camera (C-4040, Olympus). Surviving offspring were anesthetized by intramuscular injection of ketamine at 10 mg/kg into the thigh before intraoral examination, and photographs were taken using an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo, Japan).

2.4. Radiographic observation

Conventional intraoral radiographs were taken using a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto, Japan) with a charge coupled device (CCD; Gendex Visualix, Dentsply International Inc., York, PA).

2.5. Statistical analysis

All the data were analyzed using JMP5.1.1J (SAS Institute Japan, Tokyo, Japan). Analysis of variance was used to compare measurement data such as length of gestation and body weight. The incidence of tooth abnormalities was compared by using Fischer's exact probability test. A statistically significant difference was confirmed at $P < 0.05$.

3. Results

3.1. Pregnancy outcomes

TCDD administration apparently had no effect on maternal health. Pregnancy outcomes are summarized in Table 1. Abortions, stillbirths, and early postnatal deaths occurred at fairly high frequencies in the TCDD-treated groups as well as the control group. The postnatal mortality rate of the offspring was higher in the 300-ng/kg group (50%) than in the control group (28%), but the difference was not statistically significant ($P>0.1$). In an attempt to increase the number of surviving offspring in the 300-ng/kg group, we added nine dams to the group approximately 2 years after the initiation of the experiment. However, only two surviving offspring were added, due to a high incidence of abortions. There were no significant differences in the average length of gestation and average birth weight among the three groups.

Table 1
Pregnancy outcome and postnatal mortality

Dose of TCDD	No. of dams	No. of abortions	No. of stillbirths	No. of live births	No. of early postnatal deaths[a]	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	402.7±62.1
300 ng/kg[b]	9	5	1	3	1	165.0±3.0	466.0±87.1

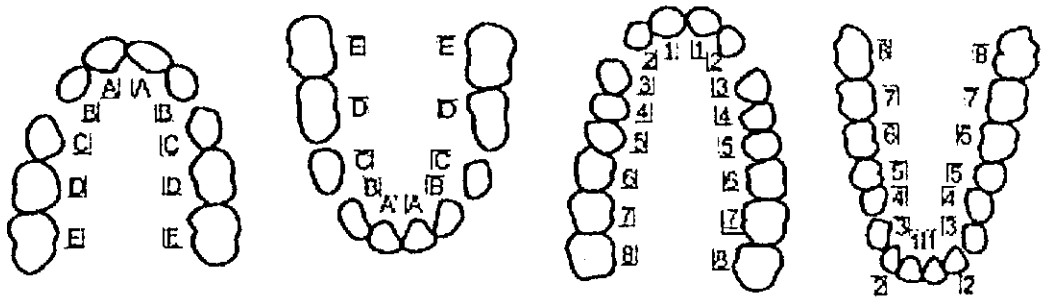
[a] Death by PND 100.
[b] Additional group.

3.2. Dental findings

3.2.1. Dentition in normal rhesus monkeys

The number and types of teeth of the rhesus monkey are similar to those of humans. The number of deciduous and permanent teeth are 20 and 32, respectively. Figure 1 illustrates outlines of these teeth and the code for designation of each tooth used in Tables 3 and 4. Neonatal monkeys usually have no erupted teeth. The central incisors erupt during the first postnatal month. The approximate ages of eruption of deciduous teeth are 1 month for the lateral

incisors, 2 months for the canines, 2.5 months for the first molars, 5 months for the second molars in the lower jaw, and 7 months for the second molars in the upper jaw. Those of permanent teeth are 2.5 years for the central incisors, 2.7 years for the lateral incisors, 3.5 years for the canines and the first premolars, 2.7 years for the second premolars, 1.5 years for the first molars, 3.5 years for the second molars, and 5.5 years for the third molars [11]. During this study we found that there were fairly large interindividual variations for the age of eruption of teeth.



Childhood upper jaw Childhood lower jaw Adult upper jaw Adult lower jaw

Fig.1 Diagram illustrating outlines of rhesus teeth seen from the occlusal plane and the code for designation used in Tables 3 and 4.
A-E: Deciduous teeth A: Central incisor B: Lateral incisor C: Canine D: First molar
E: Second molar
1-8: Permanent teeth 1: Central incisor 2: Lateral incisor 3: Canine 4: First premolar
5: Second premolar 6: First molar 7: Second molar 8: Third molar
⌋: Upper right ⌊: Upper left ⌉: Lower right ⌈: Lowere left

3.2.2. Dental findings in stillborn offspring and those that died postnatally

The incidences of tooth abnormalities are given in Table 2. During the early stage of this study, some carcasses from stillbirths and early postnatal deaths were discarded inadvertently; therefore the numbers of specimens in Table 2 are smaller than the total numbers of stillbirths and early postnatal deaths in Table 1. Stillborn fetuses from the control group had no erupted teeth in either the upper or lower jaw (Fig. 2A, E). However, conventional radiographs clearly revealed the presence of 20 well-formed deciduous teeth (Fig. 3A, E). Each tooth could be identified by its characteristic shape and size.

Dental examination of the dead offspring revealed tooth abnormalities only in the 300-ng/kg group. Three of the five animals had tooth abnormalities such as precocious eruption, dysplasia, incomplete calcification, and missing teeth. Although the incidence of tooth abnormalities in the 300-ng/kg group was high (60%), it did not differ significantly from the control incidence (0%; $P>0.1$), perhaps because of the small sample size. Descriptions of offspring with

tooth abnormalities follow, and representative macroscopic photographs and conventional radiographs are shown in Figures 2 and 3, respectively. Abnormal findings are summarized in Table 3.

Table 2
Incidence of tooth abnormalities

Group	Stillbirths and early postnatal deaths			Surviving offspring		
	No. of offspring	No. of offspring with tooth abnormalities (%)		No. of offspring	No. of offspring with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	12	0	(0)
300 ng/kg	5	3	(60)	8	6	(75*)
300 ng/kg[a]	1	0	(0)	2	1	(50)

[a] Additional group.
* Significantly different from the control group ($P<0.01$).

Table 3
Tooth abnormalities detected in stillbirths and early postnatal deaths in the 300-ng/kg group

Offspring No.	Sex	Death categories	Age[a]	Abnormal findings
34[c]	♂	Abortion	GD 128	-[b]
37[d]	♂	Stillbirth	GD 164	A|AD $\overline{A\|A}$ premature eruption, dysplasia |B missing
40[d]	♀	Early postnatal death	PND 26	|D premature eruption, incomplete calcification A|A missing
43[e]	♂	Stillbirth	GD 176	-
57[e]	♂	Early postnatal death	PND 1	BA|ABD $\overline{A\|A}$ premature eruption, incomplete calcification
103[e]	♀	Stillbirth	GD 173	-

[a] GD: gestation days; PND: postnatal days.
[b] No abnormalities were detected.
[c,d,e] Total dose of TCDD administered to the dams: [c]345, [d]360, [e]375 ng/kg.

Offspring No. 37 was stillborn on GD 164. The deciduous upper central incisors and left first molar had erupted precociously (Fig. 2B). The erupted teeth were irregular in shapes and apparently were destroyed. X-ray examination revealed incomplete calcification in the erupted teeth, and the deciduous upper left lateral incisor was missing (Fig. 3B). The deciduous lower central incisors also had erupted precociously; these teeth were dark brown

(Fig. 2F), and their calcification seemed slightly retarded (Fig. 3F) as compared with that of a control animal stillborn at an earlier gestational age (Fig. 3E, No.10: stillborn on GD 146).

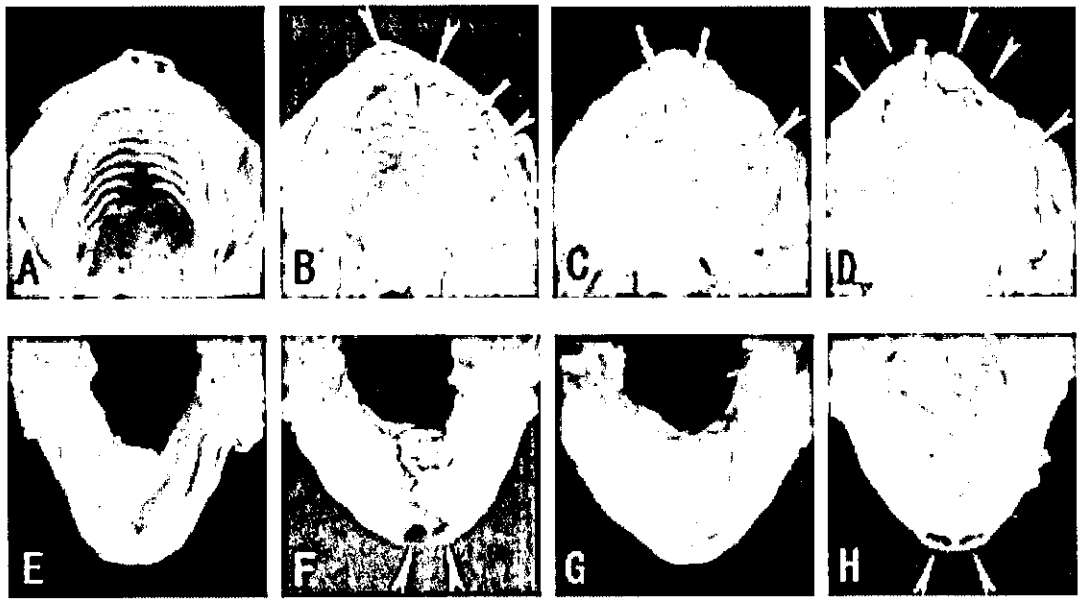


Fig. 2. Macroscopic photographs of jaws from a control offspring (A, E) and from offspring in the 300-ng/kg group with tooth abnormalities (B-D, F-H) which were stillborn or died early postnatally. Upper jaws (A-D) and lower jaws (E-H). Arrowheads indicate precocious eruption. Arrows point the location of missing teeth detected by X-ray. Offspring numbers and ages: A, E: No. 10, GD 146; B, F: No. 37, GD 164; C, G: No. 40, PND 26; D, H: No. 57, PND 1.

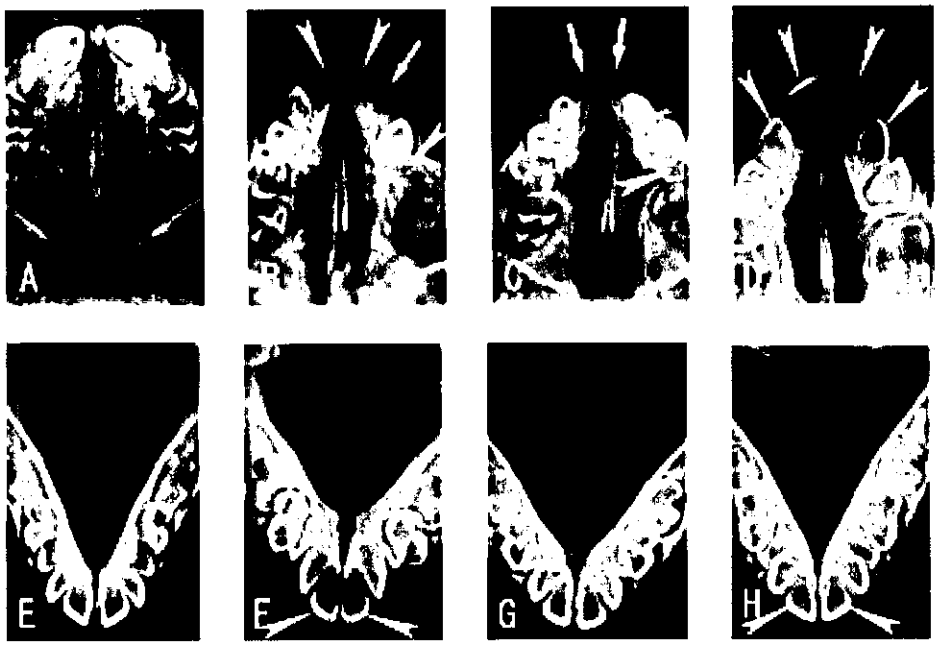


Fig. 3. Conventional radiographs of jaws shown in Fig. 2. Radiographs are arranged corresponding to Fig. 1. Upper jaws (A-D) and lower jaws (E-H). Arrowheads and arrows indicate precocious eruption and missing teeth, respectively. Offspring numbers: A, E: No. 10; B, F: No. 37; C, G: No. 40; D, H: No. 57.

Offspring No. 40 died postnatally on PND 26. The deciduous upper left first molar had erupted precociously (Fig. 2C). The four cusps were discernible macroscopically but were unclear in the radiograph (Fig. 3C), indicating retarded calcification of the tooth. X-ray examination revealed that both the deciduous upper central incisors were missing. A slight deviation of the anterior nasal septum to the left was noted (Fig. 3C). The lower teeth were still in the gum, and no abnormality was detected radiographically (Fig. 3G).

Offspring No. 57 died when a neonate, on PND 1. The bilateral deciduous upper central and lateral incisors, upper left first molar (Fig. 2D) , and bilateral lower central incisors (Fig. 2H) had erupted precociously. The lower incisors were dark brown. X-ray examination revealed retarded calcification in these precociously erupted teeth (Fig.3D, H).

3.2.3. Dental findings in surviving offspring

The incidences of tooth abnormalities in the surviving offspring are given in Table 2. The incidence in the 300-ng/kg group (total, 70%) was significantly higher than that in the control group (0%; $P<0.01$). Representative macroscopic and radiographic photographs are shown in Figures 4 and. 5, respectively. Abnormal findings are summarized in Table 4.

3.2.3.1. Offspring observed between approximately PND 800 and PND 1400

In the vehicle-treated group, offspring were at the stage of losing the deciduous teeth during the period of PND 800 to PND 1400. In the majority of animals, the permanent central and lateral incisors and the first molars had erupted. By conventional radiography, all the permanent teeth except for the third molars were detectable. Descriptions of a control animal and the monkeys from the 300-ng/kg group with tooth abnormalities follow.

Offspring No. 1 is a control animal. Figure 4A shows the central upper and lower jaws on PND 1438. The permanent central and lateral incisors as well as canines had erupted. Figure 5A is a radiograph of the anterior upper jaw taken on PND 1049. The midline is approximated by the left border of the picture. The deciduous central incisor had been lost, and the permanent central incisor had erupted. The deciduous lateral incisor still remained but was being pushed up by the growing permanent incisor. The permanent canine was discernible deep to the long root of the deciduous canine on the distal side of the root of the permanent lateral incisor. The canine could be easily identified by the pointed shape of the crown. Figure 5B shows the upper left molars radiographed on PND 1438. The deciduous first molar had been lost, and the first premolar had erupted. The crown of the deciduous second molar remained posterior to the permanent first premolar, and it was being pushed up by the permanent second premolar. The permanent first and second molars had erupted, but the third molar could not be seen clearly. Figure 5C shows the lower right lateral incisor, canine, and molars on PND 1438. The lateral incisor and canine were permanent teeth, but the deciduous first and second molars still remained, being pushed up by the growing permanent first and second premolars. The permanent first molar had erupted.

Offspring No. 31 through No. 66 are members of the 300-ng/kg group.

Offspring No. 31 was observed macroscopically on PND 1430. It was found that both the upper permanent lateral incisors were missing (Fig. 4B, arrows). The upper left second premolar had erupted but its crown was small and cone-shaped (Fig. 4C, arrowhead). These defects were confirmed by radiography (Fig. 5D, arrow; Fig. 5, E arrowhead). Radiographs taken on PND 1430 showed that the upper left deciduous first molar remained, and no permanent first premolar was found between the roots of the deciduous first molar (Fig. 5E, arrow); therefore a missing permanent first premolar was diagnosed. Similar findings from the upper right side led to diagnosis of missing first and second premolars on this side. In the lower jaw, the right second premolar had erupted, but its crown was on the

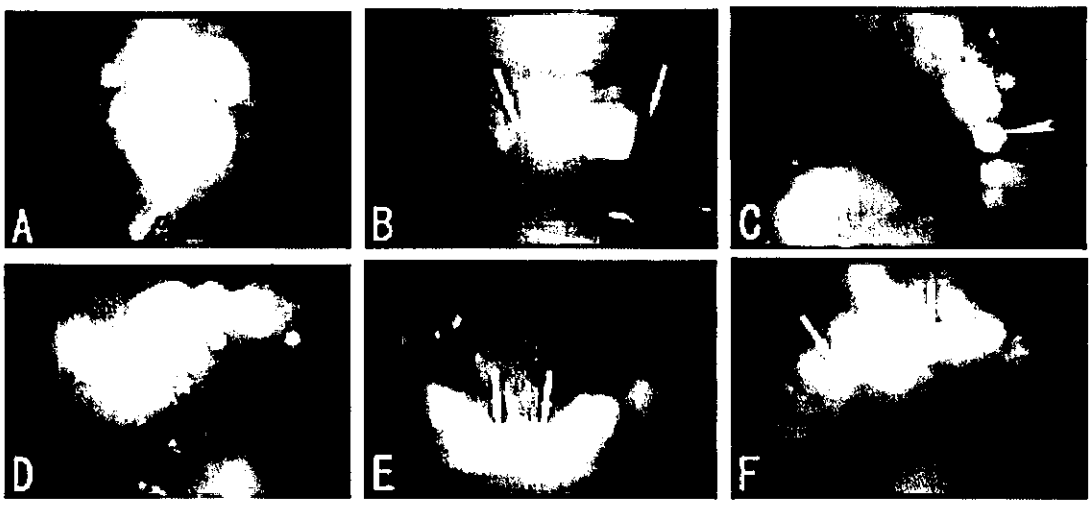


Fig. 4 Macroscopic photographs of surviving offspring in the control group (A) and 300-ng/kg group with tooth abnormalities (B-F). Arrows: missing; arrowhead: cone-shaped; star: maldirected; asterisks: remaining deciduous teeth. Offspring numbers and ages: A: No. 1, PND 1438; B, C: No. 31, PND 1430; D: No. 44, PND 1415; E: No. 66; PND 1338; F: No. 106, PND 688. A: upper and lower incisors; B: upper incisors; C: upper left molars; D: lower right molars; E: lower incisors; F: upper incisors.

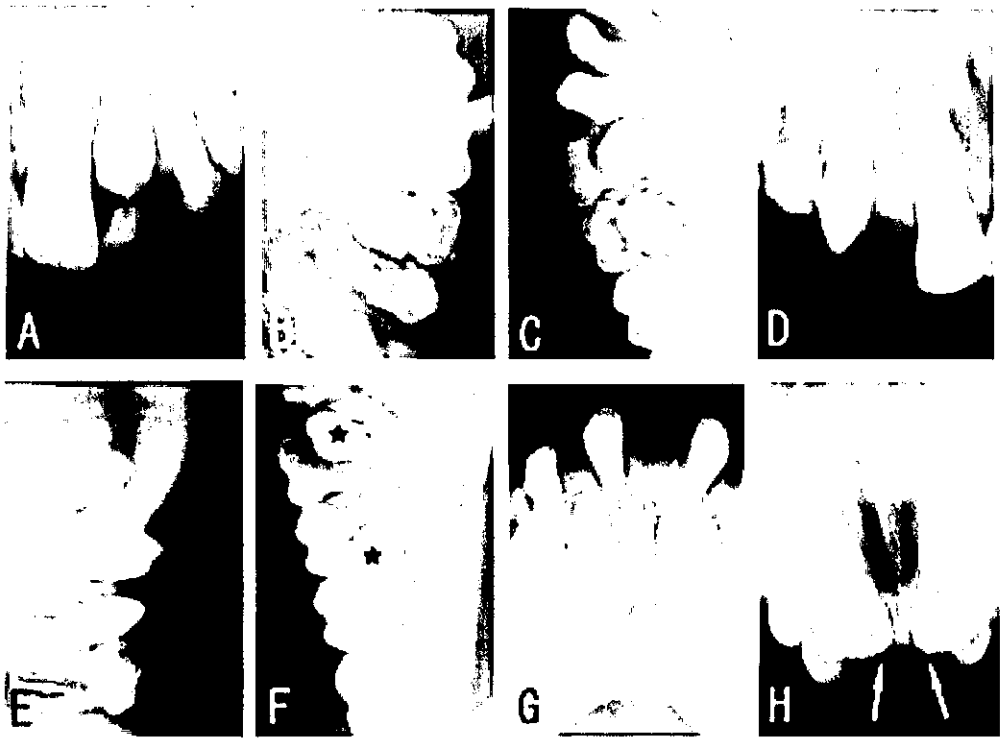


Fig. 5. Conventional radiographs of surviving offspring in the control group (A-C) and 300-ng/kg group with tooth abnormalities (D-H). Arrows: missing; arrowhead: cone-shaped; stars: maldirected. Offspring numbers and ages: A: No. 1, PND 1049; B, C: No. 1, PND 1438; D, E: No. 31, PND 1430; F: No. 44, PND 1415; G: No. 66, PND 1338; H: No. 106, PND 688. A: upper left incisors and canine; B: upper left molars; C: lower right molars; D: upper right incisor and canine; E: upper left molars; F: lower left molars; G: lower incisors; H: upper incisors

Table 4

Tooth abnormalities detected in surviving offspring in the 300-ng/kg group

Offspring No.	Sex	Age[a] at observation	Abnormal findings
31[c]	♀	941, 1041, 1122, 1430	542|24 missing, |5 cone-shped
33[d]	♂	960, 1060, 1449	-[b]
35[d]	♀	936, 1036, 1425	-
39[c]	♂	921, 1021, 1102, 1410	542|245 missing
42[d]	♀	926, 1026, 1415	5|5 missingh, |4 cone-shaped
44[d]	♂	926, 1026, 1415	$\overline{54}$|45 maldirected
60[d]	♂	899, 999, 1080, 1388	542|245 $\overline{5}$|$\overline{5}$ missing
66[c]	♂	849, 949, 1030, 1338	52|2 $\overline{1}$|$\overline{1}$ missing, |45 cone-shaped, maldirected, $\overline{54}$|45 maldirected
106[d]	♀	198, 299, 380, 688	A|A 4|24 missing
109[d]	♀	189, 290, 679	-

[a] Postnatal days.

[b] No abnormalities were detected.

[c,d] Total dose of TCDD administered to the dams: [c]420, [d]405 ng/kg.

lingual side of the alveolar gum. Radiographically, the axis of the second premolar was inclined in a lingual and distal direction.

Offspring No. 39: was examined radiographically on PND 1410. The upper permanent lateral incisors and the first and second premolars were missing bilaterally.

Offspring No. 42 had an upper left first premolar that was cone-shaped, and the upper second premolars were missing bilaterally, according to observation on PND 1415.

Offspring No. 44 had a lower right first premolar that was inclined in a lingual and mesial direction, and it had erupted on the lingual side of the alveolar gum (PND 1415; Fig. 4D, star). Because of this maldirection, the deciduous first molar still remained. Similarly, the lower right second premolar was maldirected, and the deciduous first and second molar also remained (Fig. 4D, asterisks). The lower left first premolar had not erupted, but a radiograph sowed that the first and second premolar were maldirected (Fig. 5F, stars), and the deciduous first and second molars remained.

Offspring No. 60 was evaluated macroscopially and radiographically on PND 1388. These studies indicated that both lateral incisors and both first and second premolars were missing from the upper jaw. In addition, both second premolars were missing from the lower jaw.

Offspring No. 66 had an upper jaw from which the bilateral permanent lateral incisors and the right second premolars were missing, and the left first and second premolars were cone-shaped. In the lower jaw, the permanent central incisors were found to be absent on PND 1338 (Fig. 4E, arrows). The remaining permanent incisors were close to the canines, and there was a wide space between the two incisors (Fig. 5G, arrow), indicating that the incisors were lateral ones. The upper left first and second premolars had been erupted by PND 1338, but were cone-shaped, and maldirected. The lower first and second premolars were also maldirected bilaterally.

3.2.3. 2. Offspring observed between approximately PND 200 and PND 700

The two surviving offspring that were added to the 300-ng/kg group were approximately PND 200 at the time of their first radiographic examination. They were followed until approximately PND 700. Only one of these animals, **Offspring No. 106,** had obvious tooth abnormalities. This animal had a wide space between the small incisors in the upper jaw; this defect first was observed on PND 198, and was confirmed on PND 688 (Fig. 4F, arrows). Radiographs taken on PND 688 showed the growing permanent central incisors and a wide median gap between the remaining deciduous teeth (Fig 5H, arrows), indicating that the deciduous central incisors were missing. In addition, the upper bilateral first premolars and the left permanent lateral incisor were missing.

TCDD affects tooth development in rodents. A single oral dose of 1 μg/kg to pregnant rats on GD 15 disturbed postnatal molar development in the offspring [21]. Lactational exposure through maternal intraperitoneal administration of TCDD to rats at a dose level of 1000 μg/kg on PND 1 also affected molar development in the offspring [22,23]. In addition, growing incisors in rats were sensitive to continuous exposure to TCDD for 20 weeks beginning from 10 weeks of age.[24].

Human epidemiological studies have been conducted to examine possible association between dioxin exposure and tooth abnormalities. In Finland, 102 6- to 7-year-old children who were breast-fed for an average of 10.5 months were studied. Milk samples were collected when the children were 4 weeks old, and the concentrations of dioxins and furans were determined. The total exposure to dioxins was calculated from the concentrations in milk and the duration of breast feeding. The frequency and severity of hypomineralization of teeth correlated with the total exposure [25].

Follow-up studies after accidental exposure to dioxins also have indicated that the teeth are targets of developmental toxicity of these toxicants. High frequencies of delayed eruption and missing permanent teeth occurred among children with fetal Yusho or Yuchen (oil disease), which occurred in 1968 in Japan and in 1979 in Taiwan after ingestion of rice oil contaminated with PCBs and PCDFs [6,7]. In addition, examination of 48 people exposed to dioxins because of the notorious accident in Seveso, Italy, in 1976 revealed a high incidence of developmental defects of enamel and missing permanent teeth [5]. These subjects had been younger than 9.5 years at the time of the accident and were examined for tooth abnormalities 25 years afterward. Plasma collected in 1976 had TCDD concentrations that ranged from 23 to 26,000 ng/kg in serum lipid. Subjects with higher serum TCDD levels had developmental dental defects more often than those with lower TCDD levels.

In the present study, we found positional differences among teeth as manifestations of the sensitivity to the developmental toxicity of TCDD. Even before eruption, each tooth can be easily identified in light of the position of the canine, which is large and has a characteristically pointed crown. The canines were not affected in any of our monkeys; the vulnerable teeth were the central and lateral incisors, deciduous first molars, and the first and second premolars. In the patients with Yusho, the most frequent missing tooth was the lower premolar, followed by the lower lateral incisor [6]. In humans the lateral incisor and the second premolar are considered to have a regressing tendency in the process of evolution [26], and these teeth are missing relatively frequently in the general population. This intrinsic regressive tendency might be exacerbated by exogenous toxicants, resulting in positional differences in sensitivity.

It is well known that interactions between the ectoderm covering the first branchial arch and the mesenchyme derived from the neural crest are important in tooth morphogenesis. Several signal molecules and their receptors have been identified [27]. TCDD is a potent modulator of epithelial cell growth and differentiation [28], and most of its toxic effects are mediated by the aryl hydrocarbon receptor (AhR) [29]. For example, cleft palate induction by TCDD was completely abolished in AhR knockout mice [30]. In mouse tooth buds, AhR is expressed in secretory odontoblasts and ameloblasts [23], suggesting the pathway via AhR as a mediator of dental toxicity of TCDD. One candidate for the pathway of TCDD action on tooth morphogenesis involves epidermal growth factor (EGF) and the EGF receptor (EGFR). TCDD added to cultured embryonic mandibular molar tooth germs induced depolarization of ameloblasts and disturbed morphogenesis [31]. EGF added to the TCDD-containing medium suppressed the adverse effects of TCDD. The effect of TCDD was less dramatic on tooth germs from EGFR knockout mice [31]. Although no study has assayed expression of EGF or EGFR during tooth morphogenesis in rhesus embryos, these findings suggest that the EGF-EGFR signaling system may work in tooth development in the rhesus monkey as well as the mouse and that disturbance of this system by TCDD results in dysmorphogenesis of rhesus teeth.

In addition to altered epithelial-mesenchymal interaction, excessive apoptosis may be involved in the pathogenesis of tooth defects. TCDD added to organ culture of mouse molar tooth germs did not affect cell proliferation but increased apoptosis in the epithelium, resulting in defective molar [32]. In the process of cleft palate induction in mice by TCDD, excessive apoptosis was observed in the epithelium covering the palatal processes and in the palatal mesenchyme [33]. It is plausible that apoptosis induced by TCDD played a role in induction of tooth defects in the present experiment. Cleft palate was not detected in the present study. Probably the dose levels were too low to induce cleft palate in the rhesus monkey. In the sensitive C57 BL strain of mice the LOAEL level for induction of cleft palate was reported to be 3000 ng/kg/day by oral administration during the period of organogenesis [34]. Detailed examinations of possible target organs of developmental toxicity of TCDD including the urinary, reproductive, and immune systems are in progress.

Our examination of the surviving offspring until the age of approximately 4 years revealed tooth defects only in the 300-ng/kg group. By macroscopic observation with the digital camera we could not detect mineralization defects reported in humans [5,25]. Because the permanent molars are still developing in 4-year-old rhesus monkeys, detailed further observation may reveal some subtle abnormalities such as enamel defects in the offspring currently diagnosed as normal in the 30- and 300-ng/kg groups. Blood samples taken from pregnant and lactational mothers and milk samples await analyses for TCDD concentrations. Although the dosing schedule in the present study was set to keep the body burden at 30 or 300 ng/kg, the actual maternal body burden should be assessed after the autopsy of the

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