The Effect of Perinatal TCDD Exposure on Caries Susceptibility in Rats

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the model compound of polychlorinated dibenzo-p-dioxins and furans, is a potent toxicant with the ability to hamper development. Accidental exposure to TCDD has been linked with various developmental dental aberrations in humans, and experimentally it has been shown that TCDD causes, among other defects, hypomineralization of dental hard tissues in rodents. Here, we studied the effect of very low perinatal TCDD exposure on dental caries susceptibility and mineral composition of tooth enamel in rats. Pregnant line C rats (rat line developed in our laboratory) were dosed 0.03–1.0 µg/kg TCDD on gestation day 15 and allowed to give birth and nurse until weaning on postnatal day 21. The offspring were challenged with cariogenic treatment including sugar-rich diet and nurse until weaning. The number of caries lesions in left lower molars was determined by Schiff’s staining after 8 weeks of weaning. TCDD treatment increased cariogenic treatment in the enamel at the lowest maternal dose used, 0.03 µg/kg, and at the highest maternal dose, 1 µg/kg, the lesions extended through the enamel to dentin more frequently. Changes in mineral composition measured by electron dispersive spectrometry could not explain the increased caries susceptibility. In conclusion, perinatal TCDD exposure can render rat molars more susceptible to caries.

Key Words: dioxin; TCDD; rat; caries; tooth.

Polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) are a group of notorious toxic chemicals, and also coplanar polychlorinated biphenyls (PCBs) elicit toxic responses similarly to PCDD/Fs. These ubiquitous environmental contaminants are resistant to environmental degradation and metabolism and tend to accumulate in food chains due to their lipophilic nature. The most toxic congeners of PCDD/Fs, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes many harmful effects in laboratory animals ranging from mild biochemical variations to the eventually fatal wasting syndrome (Pohjanvirta and Tuomisto, 1994). Even though the sensitivity of adult animals varies greatly, developing animals are generally affected at lower dose levels than adults. Developmental defects may be irreversible and therefore are of great concern. Gestational exposure to dioxins is minor compared with lactational exposure (Abbott et al., 1996; Li et al., 1995), but it is sufficient to induce at least the most sensitive developmental defects (Miettinen et al., 2002). Breast-fed human infants are exposed far more than adults because PCDD/Fs are mobilized from mother’s fat tissue during lactation. The daily intake of human breast-fed infants on a body weight basis may be up to two orders of magnitude higher than that of adults (80–100 pg toxic equivalent quantity (TEQ)/kg body weight vs. less than 2 pg TEQ/kg body weight) (Abraham et al., 1996; Charnley and Doull, 2005).

The effects of TCDD are mediated by a cytosolic aryl hydrocarbon receptor (AHR). Studies with AHR knockout mice show that these animals are mostly not affected by TCDD (Fernandez-Salguero et al., 1996). After TCDD binds to AHR, the ligand-receptor complex transfers to the nucleus, where it binds with AHR nuclear translocator, and the chaperones dissociate. This ligand-receptor heterodimer binds to dioxin responsive element of DNA and induces transcription of, e.g., CYP1A1 gene. However, the reason for toxic effects is poorly understood.

TCDD-induced developmental defects include well-defined hydronephrosis and cleft palate in rodents (Abbott et al., 1987), and even very low doses of TCDD alter the development of reproductive organs (Gray et al., 1997a,b; Hamm et al., 2000; Mably et al., 1992a,b; Pratt et al., 1984), bone (Miettinen et al., 2005), and teeth (Kattainen et al., 2001; Miettinen et al., 2002) in laboratory animals. Dioxin-like chemicals are reported to affect also human teeth development. High accidental perinatal exposure to a complex mixture of PCBs and PCDFs increased the number of congenitally missing tooth germs in Taiwanese children (Wang et al., 2003) and similar exposure to TCDD...
increased hypodontia in Italian children exposed to TCDD in Seveso (Alaluusua et al., 2004). Substantial dietary exposure to PCBs was associated with developmental enamel defects, demarcated opacities, and hypoplasia in humans (Jan and Vrbic, 2000) and higher end of background dioxin exposure in Finland via mothers’ milk was linked with hypomineralized enamel in molars that mineralize during the first 2 years of life (Alaluusua et al., 1999). Furthermore, the length of breastfeeding period correlated with mineralization defects in normal population (Alaluusua et al., 1996a) and with developmental defects in accidentally exposed children (Wang et al., 2003).

In rats, perinatal TCDD exposure affects teeth development in several ways: incisor eruption is accelerated, molar eruption retarded, molar size diminished, and the development of third molar can be blocked completely (Kattainen et al., 2001; Madhukar et al., 1984; Miettinen et al., 2005). Similar findings—precocious teeth, missing teeth, and incomplete calcification—were reported also in aborted and juvenile rhesus monkeys after in utero and lactational exposure (Yasuda et al., 2005). Higher doses of TCDD or hexachlorobenzene deviate growth of continuously erupting incisor in adult rats (Alaluusua et al., 1993; Long et al., 2004). Studies on the tooth structure show that chronic dietary exposure of rhesus macaques to PCBs cause metaplasia of enamel-secreting ameloblast in unerupted molars (McNulty, 1985), TCDD increases apoptosis in murine dental epithelium in vitro (Partanen et al., 2004), and postnatal TCDD exposure infringes on enamel maturation and decelerates dentin mineralization in rats (Gao et al., 2001). Occupational PCB exposure of mothers has been linked with carious primary teeth in children (Hara, 1985), and accidental exposure increased tooth chipping in Taiwanese Yucheng subjects (Rogan et al., 1988). However, only minor (Rogan et al., 1988) or no correlation between TCDD or PCB/PCDF exposure and caries were found in Seveso or Yucheng children (Alaluusua et al., 2004; Wang et al., 2003). Because human studies have given controversial results, it is of interest to verify experimentally whether dioxin exposure can increase caries susceptibility in a controlled animal study. Indeed, preliminary studies suggested that rats exposed to TCDD perinatally had more smooth surface dental caries than controls (Bowen et al., 2001). Caries is a multifactorial oral disease in which acids—produced predominately by mutans streptococci metabolism—demineralize teeth, which results in dental cavities. The purpose of the current study was to scrutinize whether perinatal TCDD exposure increases caries susceptibility in rats and whether the possible increase is due to TCDD-induced changes in the mineral composition of the tooth enamel.

### MATERIALS AND METHODS

**Chemicals.** TCDD was >99% pure as determined by gas chromatography–mass spectrometry. TCDD was weighed and dissolved in diethyl ether for storage. Adjusted volume of diethyl ether was mixed with corn oil (Sigma Chemicals, St. Louis, MO), and the ether was let to evaporate. Dosing solutions were mixed in a magnetic stirrer and sonicated for 20 min before dosing.

**Animals.** Line C rats were obtained from the SPF barrier unit of the National Public Health Institute (Kuopio, Finland). Line C rats originate from selective cross-breeding of Han/Wistar and Long-Evans rats, have a normal AHR, and LD50 for females is 19 μg/kg (Tuomisto et al., 1999). Pregnant dams were kept in plastic Macrolon cages containing aspen-chip bedding (Tapevi Co., Kaavi, Finland) and covered with wire-mesh lids until the pups were born and weaned. After weaning on postnatal day (PND) 21, the animals were kept with same-sex littermates in wire-mesh bottom stainless steel cages. The rats were kept under a photoperiodic cycle of 12 h light/12 h dark in an air-conditioned room. The mean temperature and the relative humidity were 21 ± 0.2°C and 50 ± 3%, respectively. Pelleted rat feed (R36, Lactamin, Stockholm, Sweden) and tap water were available ad libitum for dams and pups before weaning. After weaning the rats were given either normal rat powder feed (R36, Lactamin) and tap water or sugar-rich diet consisting of rat powder feed containing 15% added sugar and tap water with 7% w/w sugar (99.9% sucrose by Suomen Sokeri, Kantvik, Finland). Third molar samples assigned for mineral composition studies were obtained from a previous study (Kattainen et al., 2001) with similar exposure scheme, but the diet was normal pelleted rat feed and tap water after weaning.

**Experimental design.** The treatment groups, number of animals, and litters are presented in Table 1. Rats were mated overnight 1–2 females with

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Maternal TCDD dose (μg/kg)</th>
<th>Streptococcus mutans</th>
<th>Diet</th>
<th>Number of studied animals/number of studied litters</th>
<th>Weight, g (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>0</td>
<td>–</td>
<td>Normal</td>
<td>48/10</td>
<td>240 ± 8</td>
</tr>
<tr>
<td>C-2</td>
<td>0</td>
<td>+</td>
<td>Sugar rich</td>
<td>42/8</td>
<td>254 ± 9</td>
</tr>
<tr>
<td>C-3</td>
<td>1</td>
<td>–</td>
<td>Normal</td>
<td>12/3</td>
<td>253 ± 8</td>
</tr>
<tr>
<td>0.03 μg/kg</td>
<td>0.03</td>
<td>+</td>
<td>Sugar rich</td>
<td>29/6</td>
<td>278 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1 μg/kg</td>
<td>0.1</td>
<td>+</td>
<td>Sugar rich</td>
<td>25/6</td>
<td>260 ± 12</td>
</tr>
<tr>
<td>0.3 μg/kg</td>
<td>0.3</td>
<td>+</td>
<td>Sugar rich</td>
<td>24/5</td>
<td>290 ± 5&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 μg/kg</td>
<td>1</td>
<td>+</td>
<td>Sugar rich</td>
<td>32/7</td>
<td>236 ± 9</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05 compared to C-2.
<sup>b</sup>p < 0.05, one-way analysis of variance with least significant difference compared to C-1.
1 male and the day sperm was confirmed in vaginal smear was assigned pregnancy day 0. Pregnant dams were given a single oral dose of 0.03, 0.1, 0.3, or 1 μg/kg TCDD on gestation day (GD) 15 at a volume of 4 ml/kg. Rats in control groups C-1 and C-2 received corn oil, C-3 were exposed to 1 μg/kg TCDD but not to cariogenic diet. The offspring number was recorded and litter size adjusted to six littersmates on PND 1, if possible.

The mouths of offspring assigned to cariogenic diet (groups C-2, 0.03 μg/kg, 0.1 μg/kg, 0.3 μg/kg and 1 μg/kg, Table 1) were inoculated with 0.5 ml of fresh 20-h broth culture of Streptococcus mutans (ATCC 27351, American Type Culture Collection, Manassas, VA) on PND21, PND22, and PND40; animals in control groups C-1 and C-3 received the same volume of clean vehicle. The offspring were killed by CO2 asphyxiation and cervical dislocation on PND77.

The body weight gain of the pregnant dams during pregnancy and body weight of offspring on PND0 was not affected by TCDD exposure. The highest dose lowered survival of the offspring; 50 and 58% of group C-3 and 1-μg/kg animals were alive on the day of termination as compared to 83 and 95% in groups C-1 and C-2. In other TCDD-treated groups, the number of survivors was similar as in control groups C-1 and C-2, i.e., 81–97% of animals. Eighty-five percent of mortality occurred perinatally, and the rest died during the first week after weaning. The body weight and body weight gain after weaning of the rats fed with cariogenic diet were generally greater than those of control rats at the age of 11 weeks except for rats exposed to a maternal dose of 1 μg/kg TCDD that had similar body weight with C-1 rats (Table 1). Male rats in the group 0.3 μg/kg were significantly heavier than C-2 males, and female rats in groups C-1, C-3, and 1 μg/kg weighed significantly less than C-2 rats at the age of 11 weeks.

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### RESULTS

#### Caries Scoring and Number of Molars

Caries scoring was performed by an experienced examiner, using a magnifying glass (63x) and a probe (0.4 mm). The presence of all the four third molars was confirmed using dissection microscopy. The left side of the lower jaws was hemisectioned sagittally and stained with Schiff’s reagent for caries scoring according to König (1966). Fissure caries was graded in two severity classes: in T lesions the caries had reached the dentinoenamel junction, and in B lesions the dentin was involved. One examiner (H.M.M.) blinded to the treatment group scored the lesions from five fissures. Two fissures in the first and second molars and one fissure in the third molar on the left side were included in the analysis.

#### Enamel and Dentin Mineral Composition

Enamel and dentin mineral composition of the lower third molars was studied from five males per group using electron probe microanalyzer (EPMA). Because in this study all lower third molars were missing from all rats exposed to 1 μg/kg TCDD, enamel mineral composition from additional rats from a previous study with missing lower third molars in only 60% of males (Kattainen et al., 2001) was analyzed using scanning electron microscopy (SEM) and energy-dispersive spectrometry (EDS). These rats were otherwise treated as those of the present study, but they were not subjected to the cariogenic treatment and they were killed at the age of 70 days. The organic material was removed, and the jaws were fixed in 10% neutral formaldehyde (previous study) or in 9% ethanol (caries study) at room temperature. For SEM the third molars were dissected out and dehydrated through a graded ethanol series. The third molars from both studies were embedded in plastic (EpoFix Kit, Struers, Denmark) according to the manufacturer’s instructions using brass ring as a mold. The molars were ground transversally through the crown in order to have both enamel and dentin in the ground section. The surface was polished with sanding papers (Exakt, Oy Algold Ab, Espoo Finland) up to grit 2000 (caries study) or 4200 (previous study), and the surface was coated with carbon.

**Electron probe microanalyzer.** A Jeol JXA-8200 superprobe WDS/EDS EPMA (Jeol, Tokyo, Japan) with five spectrometers and 10 crystals was used to evaluate the degree of mineralization. Four samples from different depths of the enamel and three samples of the dentin were analyzed from each tooth, and the average was used as a result. In each sample, the concentrations (wt%) of Ca, P, Cl, O, Na, Mg and the total mineral content were quantified. The EPMA operating conditions were 15 kV acceleration voltage and a working distance of 15 mm.

**SEM and EDS.** SEM analyses were performed with a Jeol JSM-6400 microscope (Jeol) as described previously (Koivukangas et al., 2002). Backscatter electrons (BSE) images were used to evaluate the degree of mineralization. To distinguish regions between dentin and enamel, SEM images were obtained using BSE. In this method, the BSE signal is converted into a digital gray-scale image, where the intensity (gray level) of any pixel in the image is proportional to the mean atomic number of the corresponding location on the target material (Koivukangas et al., 2002). BSE images were collected at 2664 × 2000 pixel resolution with 256 gray levels. To ensure the stability of the instruments, the BSE images were calibrated using a cobalt standard. One BSE image at ×300 magnification was collected from the cross section of the tooth crown, and three spectrums were analyzed from different zones of enamel. The concentrations (wt%) of Ca, P, Cl, O, Na, and the total mineral content in each sample were quantified using EDS (INCA 3.03; Oxford Instruments, Witney, UK). The SEM operating conditions were 15 kV acceleration voltage and a working distance of 15 mm.

#### Statistics

Due to missing third molars, the data were analyzed both including and excluding third molars. The results were similar in both analyses; therefore, the data are presented including the third molars. Animal weight and total weight of consumed water and feed were tested using one-way analysis of variance followed by the least significant difference test. If the variances between groups differed significantly, nonparametric Kruskal-Wallis followed by Mann-Whitney U was used. The proportions of animals with four third molar, caries lesions or T lesions only were tested using Fisher’s exact test.

#### Body Weight and Survival

The body weight gain of the pregnant dams during pregnancy and body weight of offspring on PND0 was not affected by TCDD exposure. The highest dose lowered survival of the offspring; 50 and 58% of group C-3 and 1-μg/kg animals were alive on the day of termination as compared to 83 and 95% in groups C-1 and C-2. In other TCDD-treated groups, the number of survivors was similar as in control groups C-1 and C-2, i.e., 81–97% of animals. Eighty-five percent of mortality occurred perinatally, and the rest died during the first week after weaning. The body weight and body weight gain after weaning of the rats fed with cariogenic diet were generally greater than those of control rats at the age of 11 weeks except for rats exposed to a maternal dose of 1 μg/kg TCDD that had similar body weight with C-1 rats (Table 1). Male rats in the group 0.3 μg/kg were significantly heavier than C-2 males, and female rats in groups C-1, C-3, and 1 μg/kg weighed significantly less than C-2 rats at the age of 11 weeks.

#### Water and Feed Consumption

Consumption of feed was decreased in male rats that were on cariogenic diet, whereas in females feed consumption was similar between all the groups. The rats drinking sugared water consumed clearly more water than those drinking plain water. Total water consumption at the end of the feeding period was around 2–3 kg/animal in groups with cariogenic diet, whereas C-1 control rats had consumed only about 1 kg/animal water, indicating that rats prefer to drink sugared water.

#### Missing Third Molars

Animals without four developed third molars were found only in groups exposed to TCDD (Table 2). Two animals (8%) in the group 0.1 μg/kg missed one lower third molar, whereas all upper third molars were developed. Every offspring exposed to 1 μg/kg (groups C-3 and 1 μg/kg) lacked all the lower third molars.
molars. Of animals in groups C-3 and 1 μg/kg, 58 and 66%, respectively, missed one or two upper third molars.

Caries

In animals exposed to cariogenic diet, TCDD increased the number of caries lesions (Fig. 1) as compared to control group C-2 (Fig. 2). The number of B lesions involving dentin increased statistically significantly at the highest maternal dose 1 μg/kg, and consequently, the percentage of animals with T lesions exclusively was decreased at this dose level (Table 2). TCDD treatment increased the percentage of animals with caries lesions from 60% in control group C-2 to 91% at the highest dose level (Table 2). Furthermore, 1 μg/kg TCDD alone without cariogenic treatment caused caries, though only in one animal, in the C-3 group (Fig. 2). In all groups with cariogenic treatment the total number of caries lesions was greatest in the second molars (Fig. 2) and the number of caries lesions per fissure was lowest in the first molars (data not shown). The presence of S. mutans was similar in all groups challenged with cariogenic treatments, with the exception of significant increase in the 1-μg/kg group (data not shown). The numbers of caries lesions in males and females did not differ and are therefore presented together.

Enamel and Dentin Mineral Composition

In the previous dose-response study, 50% of females and 40% of males had developed lower third molars. Mineral composition in the enamel was analyzed in these rats from the developed third molars (Fig. 3). The weight percentage of calcium was dose-dependently diminished, and the reduction was statistically significant in the 1-μg/kg-dose group compared to controls.

![FIG. 1. Second molar of rats exposed either to normal (group C-1, top) or cariogenic diets (group 0.1 μg/kg, below). Note fissure caries (arrows) and approximal caries (arrowhead).](https://academic.oup.com/toxsci/article-abstract/91/2/568/1656718)

![FIG. 2. Mean number of T and B lesions (caries involving dentinoenamel junction and dentin, respectively) in rats (upper) and number of caries lesions per each molar (lower). Mean ± SE. * means that the total number of caries lesions is p < 0.05 versus C-2, C-1 means p < 0.05 versus C-1; Mann-Whitney U-test. Note that there were no developed third molars at the dose 1 μg/kg.](https://academic.oup.com/toxsci/article-abstract/91/2/568/1656718)
In rats subjected to cariogenic treatment, the relative mineral contents of third molar enamel were altered minimally at dose levels 0.03–0.3 μg/kg. No developed third molars were available at dose level 1 μg/kg in rats subjected to cariogenic treatment. The only mineral, whose average value for the four measurement points decreased significantly, was magnesium oxide at the dose level 0.3 μg/kg as compared to C-1 and C-2, and there appeared to be an inverse gradient of Mg in the exposed rats in comparison to control rats (Fig. 3). The two major components of measured elements, calcium oxide and phosphorus oxide, did not show any alterations in their relative amounts that were 48.8–49.4% and 38.7–39.1% in enamel at and below dose level 0.3 μg/kg, respectively. There were no alterations in the average relative amounts of minerals in the dentin. Calcium oxide and phosphorus oxide ranged from 33.9 to 35.3% and 41.5 to 42.8%, respectively, in the dentin at dose levels 0.03–0.3 μg/kg.

**DISCUSSION**

The objective of this study was to determine whether perinatal TCDD exposure affects susceptibility to dental caries in rats exposed in utero and via lactation. Previous studies have revealed that two mineralized tissues, teeth and bone, are both affected after low gestational and lactational TCDD exposure in rats and that the development of molars belongs to the most sensitive end points of dioxin toxicity (Kattainen et al., 2001; Miettinen et al., 2002, 2005). The lowest dose used in this study is sufficiently high to diminish rat molar size (Kattainen et al., 2001); yet, assuming linear correlation for dose and tissue concentration and an equal distribution of dioxin in the lipid compartment of the body results in a maternal fat tissue concentration of 150 pg TCDD/g lipid (Miettinen et al., 2005) that is comparable to a high end concentration 100 I-TEQ pg/g lipid measured in Finnish human breast milk in 1980s (Alaluusua et al., 1996b). The highest dose affected the postnatal survival in the used rat line and blocked the development of the third molars in the present study being in line with our previous studies (Miettinen et al., 2002). The dosing day GD15 targets different developmental stages of molars. First molars are at bud stage on GD15 and second molars are just initiated, whereas the third molars are yet to initiate on GD20. The dosing occurred clearly before the start of mineralization commencing on GD20, PND1–2, and PND13–14, respectively, for first, second, and third molars (Shellis and Berkovitz, 1981).

TCDD dosing increased caries susceptibility in rats challenged with cariogenic treatment. The sensitivity increased at the lowest tested dose, 0.03 μg/kg, and was comparable within groups 0.03, 0.1, and 0.3 μg/kg. Approximal caries that indicates high caries activity was detected frequently even if it was not systematically studied. This result strengthens the view that teeth development is affected at low doses relevant for risk assessment. At the highest dose, 1 μg/kg, the severity of caries was notably greater than in C-2 group and other TCDD groups, as seen by increased number of caries lesions involving dentin. TCDD dosing also increased the proportion of animals with caries. Interestingly, second and third molars were more susceptible to caries than the first molars. This is in line with our previous results (Miettinen et al., 2002), indicating that exposure even before tooth initiation affects tissues or their interactions so that the forthcoming molar is more vulnerable for the toxic effects. One animal in the C-3 group, that received no cariogenic diet, was determined to have a T lesion. Our results are in line with a preliminary study (abstract) from Bowen et al. (2001), who found that a maternal TCDD dose of 0.06 μg/kg on GD8 increased smooth surface caries. Some human studies suggest that dioxin-like compounds might have cariogenic effect (Hara, 1985; Rogan et al., 1988), but not all studies have found a correlation (Alaluusua et al., 2004). Occupationally PCB-exposed mothers self-reported carious teeth in their children with increasing frequency in correlation with breast-feeding time, but a health examination could not confirm increased caries in relation with children’s blood PCB level (Hara, 1985). A possible explanation for controversy between rat and human results is oral hygiene combined with species differences. Altogether, the present study shows that

![FIG. 3. Mineral contents in enamel. (a) Relative average content of calcium from the previous dose-response study (Kattainen et al., 2001), * means p < 0.05 versus control group; (b) relative amounts of magnesium in different measurement points.](https://academic.oup.com/toxsci/article-abstract/91/2/568/1656718/572)
increased caries susceptibility due to perinatal TCDD exposure is biologically possible.

The mechanism by which TCDD increases caries susceptibility could be altered enamel and dentin structure or deviated mineralization. A higher dose of TCDD, 50 μg/kg, to lactating dams interfered with organic matrix removal in offspring enamel and detained mineralization of dentin (Gao et al., 2004). The effect appears to be permanent since the organic matrix of the enamel was detected in the erupted teeth that are not mineralized any more. Additionally, TCDD treatment resulted in the failure of enamel deposition and mineralization of dentin matrix in embryonic mouse teeth in vitro (Partanen et al., 1998). In humans, broken or chipping teeth have been linked with PCDD/Fs or PCBs exposure (Guo et al., 1999; Rogan et al., 1988). Because TCDD exposure diminishes teeth size (Kattainen et al., 2001; Miettinen et al., 2002), it is likely that the enamel layer is also thinner. Therefore, caries could reach dentin more rapidly in the TCDD-exposed animal teeth. The TCDD-induced effects on mineralization are not limited to teeth because TCDD decreased bone mineral density in rats after perinatal exposure in rat offspring (Miettinen et al., 2005) and altered dimensions and mechanical parameters of bones in adult rats (Jääsa et al., 2001). TCDD exposure affects bone development also in fish (Hornung et al., 1999). The relative amount of calcium in the enamel of TCDD-treated rats was significantly decreased at dose level 1 μg/kg. However, at low dose levels relative alterations in mineral composition do not explain increased caries susceptibility.

Saliva flow sweeps plaque from teeth and neutralizes acids produced by plaque bacteria, thus decreased saliva secretion can affect caries susceptibility. Furthermore, salivary antibodies are important caries repressors. TCDD interferes with murine salivary gland development in vitro (Kiuukkanen et al., 2005) and plausibly also in vivo. In the Seveso subjects, salivary flow was not changed. However, the subjects had not been exposed during salivary gland development, whereas in the present study, TCDD might have hampered salivary gland development. Salivary glands, similar to other epithelial appendages, develop via branching morphogenesis, which is altered by TCDD at least in ventral prostate and mammary glands (Fenton et al., 2002; Ko et al., 2002).

First and second molars of rats normally erupt on PND16 and PND17 (Menaker and Navia, 1973), respectively, while juvenile rats are still lactated by the dam. Because TCDD retards rat third molar eruption (Kattainen et al., 2001) and rats were weaned to cariogenic diet and inoculated with S. mutans on PND21, it is possible that in TCDD-exposed groups second molars erupt around the time of cariogenic challenge. This might render molars of exposed rats more vulnerable to caries because teeth are susceptible to caries around eruption, because the maturation of the enamel is still in progress. Previously we have shown that the sensitivity of developing dentition for TCDD increases mesiodistally. This appears to hold true for caries also because the number of caries lesions per fissure, i.e., initiation and detection site of caries, was lowest for first molars and similar in the second and third molars in animals with developed third molars.

Even if enamel is the first barrier confronting caries, we are unaware of reports dealing with the effect of sucrose on the development of enamel. The metabolism of dentin might be a more important determinant of caries susceptibility than the structure of enamel (Larmas, 2003). High-sucrose diet alters dentin development in juvenile rats, which presumably affects caries susceptibility. Sucrose-rich diet after weaning reduced the amount of calcium, phosphorus, and total minerals in rat molar dentin compared with the preweaning time, and total minerals were decreased slightly but not significantly compared with controls receiving normal diet (Huumonen and Larmas, 1999). The possible influence of sucrose on dentin composition and thereby on susceptibility to caries was controlled in the present study by using a control group with a diet similar to that of the TCDD-treated group.

A further mechanism behind increased caries susceptibility could be TCDD-induced alterations in immune defense because caries is infectious by nature. Adult animals respond to TCDD treatment with suppressed infection resistance (IARC, 1997), and perinatal TCDD exposure causes alterations in immune functions. For example, maternal dose 1 μg/kg permanently suppressed delayed-type hypersensitivity in the offspring (Gehrs et al., 1997). Both salivary (IgA) and serum-derived (IgG) antibodies protect against the adherence of mutants streptococci and interfere with streptococci metabolism (Tenovuo and Aaltonen, 1991). The importance of normal immune defense is represented by the fact that homozygous (rnu/rnu) mice that are congenitally athymic are more susceptible to caries than normal rats (rnu) (Stack et al., 1990).

Epidermal growth factor signaling is involved in dioxin-induced dental toxicity (Partanen et al., 1998); yet, AHR is probably the initiating mediator of toxicity. AHR is expressed in the specialized cells of teeth, enamel-forming ameloblasts and dentin-forming odontoblasts. TCDD decreased AHR expression in secretory ameloblasts and odontoblasts in the offspring lactated by TCDD-treated rat dam (Gao et al., 2004).

The agenesis of third molar is a highly reproducible effect at the maternal dose level 1 μg/kg (Kattainen et al., 2001; Miettinen et al., 2002; present study). Previously 50% of female offspring and 60% of male offspring have had less than two lower third molars, when the dam has been exposed on GD15. However, in the present study exposure on GD15 resulted in 100% frequency in lower third molar agenesis. This is the first study to report missing molar induced by TCDD detected in rat offspring below the maternal dose 1 μg/kg. Two siblings at the dose level 0.1 μg/kg, that did not affect offspring weight, missed one lower third molar each. Molar agenesis has been reproduced experimentally in rhesus monkeys, though on a toxic dose (Yasuda et al., 2005), and teeth agenesis was found in exposed rainbow trouts (Hornung et al., 1999). Missing teeth have been reported in studies of accidentally exposed humans
(Wang et al., 2003). In Seveso, hypodontia was detected in 13% of children less than 9.5 years of age at the time of exposure (Alaluusua et al., 2004), as other symptoms of TCDD toxicity in Seveso children have been confined to acute dermatotoxic effects such as chloracne (Baccarelli et al., 2002). Collectively, the data implicate that dioxin and dioxin-like substances are able to prevent tooth development and in rats even at dose levels that do not cause other severe developmental effects.

In summary, perinatal exposure to TCDD enhances caries susceptibility in rats. The effect was observable already at the lowest dose of 0.03 µg/kg TCDD and was more severe at the highest dose level 1 µg/kg. The enhanced susceptibility could not be explained by altered relational amounts of calcium. At doses less than 1 µg/kg, other factors such as diminished salivary flow related to developmental salivary gland toxicity of TCDD could be involved. The results strengthen the view that developing teeth belong to the highly sensitive target organs of TCDD toxicity.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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