Molar-Incisor-Hypomineralisation and Dioxins: New Findings

S. Laisi,**, H. Kiviranta***, P-L Lukinmaa+,++, T. Vartiainen***, S. Alaluusua**,#*
**Health Center, Ylämaa, **Dept. of Pediatric and Preventive Dentistry, Institute of Dentistry, University of Helsinki; ***Dept. of Environmental Health, National Public Health Institute, Kuopio; +Dept. of Oral Pathology, Institute of Dentistry, University of Helsinki, Helsinki, ++Dept. Pathology, Helsinki University Central Hospital, Helsinki, #Dept. Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki; Finland*

Abstract

Aim: According to our earlier study, molar-incisor hypomineralisation (MIH) was associated with the exposure of a child via mother's milk to polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) in a group of Finnish children born in 1987. Since the levels of PCDD/Fs and PCBs in mother's milk/placenta have remarkably decreased, it was important to find out if an association still exists. **Methods:** The study group was composed of 167 mothers and their children. Placental samples from the mothers were collected in maternity hospitals in Helsinki and Oulu in 1995-1999 and concentrations of the 17 most toxic PCDD/PCDF and 36 PCB congeners were measured. After 7-10 years the children were examined for MIH and the mothers were interviewed on the duration of breast-feeding. **Results:** MIH was found in 24 children (14.4%). The duration of breast-feeding ranged from 0 to 30 months (mean=7.2±4.7). $WHO_{PCDD/F}$TEQ ranged from 2.5 to 39.1 pg/g fat (mean=13.7±6.8) and WHO_{PCB}TEQ from 0.7 to 9.8 pg/g fat (mean=2.7±1.4). The mean sum of PCDD/Fs was 196±105 pg/g fat and that of PCBs was 57.2±28.1ng/g fat. The total exposure to PCDD/Fs, which was calculated from the placental concentration (used as a proxy for the milk concentration) and duration of breast-feeding, was not associated with the occurrence or severity of MIH. Neither was the total exposure to PCBs associated with the occurrence or severity of MIH. **Conclusion:** At prevailing levels, exposure of a child via placenta/mother's milk to PCDD/Fs and PCBs is not associated with MIH.

Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), collectively called dioxins, and polychlorinated biphenyls (PCBs) are widely spread environmental pollutants. They accumulate in the food chain and are secreted in human milk. Their adverse health effects on different species, including humans, are unequivocal (Environmental Protection Agency, U.S.A.).

Tooth development is genetically regulated but sensitive to environmental effects. Many developmental disturbances may become ameliorated with time, but as dental hard tissues are not renewed, these faults remain permanent. During the period of development of the permanent dentition children can be exposed to compounds that may harm tooth development transplacentally via mother's milk, and through diet.

Experimental studies on rhesus monkeys and laboratory rodents in vivo and in vitro show that dioxins and PCBs disturb tooth development [Alaluusua and Lukinmaa, 2006]. The effects of the most toxic dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), depend not only on the dose but also the stage of tooth development. Accordingly, TCDD totally arrests early developmental stages of rat and mouse molar teeth [Lukinmaa et al., 2001; Kattainen et al., 2001; Partanen et al., 2004] and exposure during later developmental stages results in delayed and defective mineralisation of the dental matrices and an arrest of root development [Lukinmaa et al., 2001; Gao et al., 2004].

In agreement with the results of animal experiments, children who were accidentally exposed to high amounts of PCDDs, mainly TCDD, in Seveso, Italy, in 1976, had more developmental enamel defects and missing permanent teeth than the control children [Alaluusua et al., 2004]. In the Yu-Cheng accident in Taiwan in 1978, about 2,000 people were exposed to cooking oil contaminated with PCBs and PCDFs. Exposed children also had more missing teeth and dental defects than their controls [Wang et al., 2003]. In accordance with previous studies, children in Slovakia exposed to higher amounts of PCBs, originating in a chemical plant that had contaminated the surrounding district, had more often and more severe developmental enamel defects in their permanent teeth than children who were exposed to lower concentrations [Jan et al., 2007].

In an earlier study the dentitions of a cohort group of healthy Finnish children born in 1987 were examined, whose mothers had taken part in a WHO/EURO coordinated follow-up study on the levels of PCDD/Fs in breast milk [Alaluusua et al., 1996; Alaluusua et al., 1999]. Concentrations of the 17 most toxic PCDD/F and 33 PCB congeners in milk samples,

Key words: Dioxins, PCDD, PCDF, PCB, teeth, human milk, placenta, and developmental dental defect

Postal address: Prof. S. Alalusuua, **Dept. Pediatric and Preventive Dentistry, Institute of Dentistry, University of Helsinki, P.O. Box 41, FI-00014, Helsinki; Finland.

Email: Satu.Alaluusua@helsinki.fi

collected from mothers when these children were 4 weeks neonate were determined [Vartiainen et al., 1997]. An association was found between dioxin exposure via mother's milk and developmental enamel defects in the first permanent molars (FPMs). In the same study the sum of international TCDD-equivalent quantity (I-TEQ) for PCDD/Fs ranged from 3.8 pg/g to 99 pg/g fat and PCB-TEQ from 3.7 pg/g to as high as 162 pg/g fat in the milk samples. Finally, results of animal studies were in line with those of observations in humans: a single dose as low as 30-100 pg/g TCDD to the rat dam on gestation day 15 led to disturbances in molar tooth development [Kattainen et al., 2001]. These doses were found to produce maternal adipose tissue concentrations [Hurst et al., 2000] that did not essentially differ from those at the high end of the range of human milk fat concentrations in late 1980's in Finland.

The levels of PCDD/Fs and PCBs in mother's milk have remarkably decreased during the last decades [Leeuwen and Malisch, 2002; Kiviranta, 2005]. The annual decrease in TEQs of PCDD/Fs and PCBs in Finland from mid 1980's to mid 1990's was 4% and 8%, respectively.

The aim of the present study was to a) examine the existence of an association between developmental enamel defects in the permanent first molars, currently called molar-incisor hypomineralisation (MIH), [Weerhejm et al., 2001], and the exposure to PCDD/Fs and PCBs in early childhood and b) to compare the present results with those reported ten years before [Alaluusua et al., 1996].

Materials and methods

Study group. The children and their mothers included in the present research participated in a larger study in which the role of dioxins on cleft lip and cleft palate will be evaluated. The mothers who gave birth to a child with a cleft between 1995 and 1999 and a group of control children were recruited from maternity hospitals in Helsinki, Turku, Oulu and Kuopio (Finland). Placental samples were obtained from 91 mothers who had a child with a cleft and from 374 controls. Of those 465 cases, 167 children and their mothers living in Helsinki and Oulu districts participated in the present study. There were 28 children with a cleft.

Analysis of placental samples. Whole placentas, collected by the midwives and immediately frozen in polyethylene bags (–20°C), were transported to the laboratory. On receipt the samples were defrosted and the umbilical cord and all readily removable membranes were discarded. Whole placentas were homogenised and 75 g of the homogenate was lyophilised. Dried homogenate was pulverised in a mortar and a slurry was made by adding dichloromethane and cyclohexane (1:1 v/v) and concentrated sulphuric acid. This slurry was spiked with sixteen ^{13}C-labeled PCDD/F standards (2,3,7,8-chlorinated PCDD/F congeners), with three ^{13}C-labeled non-*ortho*-PCB standards (PCB 77, 126, and 169), and nine other ^{13}C-labeled PCB standards (PCB 30 [^{12}C-labeled], 80, 101, 105, 138, 153, 156, 180, and 194), (Wellington Laboratories Inc., Guelph, Canada). The occurrence of 17 PCDD/F (toxic) congeners, of three non-*ortho* (PCB 77, 126, and 169), eight mono-*ortho* (PCB 105, 114, 118, 123, 156, 157, 167, and 189), of 25 (PCB 18, 28/31, 33, 47, 49, 51, 52, 60, 66, 74, 99, 101, 110, 122, 128, 138, 141, 153, 170, 180, 183, 187, 194, 206, and 209) other PCB congeners were measured. Analyses of PCDD/Fs were successful for all 167 placentas, but with PCBs there was a loss of 13 placenta analyses in the laboratory due to human error during the analysis.

The procedure for decomposition of fat and sample clean up has been described in detail previously [Kiviranta, 2004]. In short, a placenta-solvent-sulphuric acid slurry was laid on top of a silica gel column containing acidic and neutral layers of silica and eluted in order to decompose fat from the sample. After fat removal PCDD/Fs were separated from PCBs on activated carbon column. The two fractions including PCBs and PCDD/Fs were further cleaned by an activated alumina column after which another activated carbon column was used for PCBs in order to separate the non-ortho PCBs from other PCBs. Quantitation was performed by selective ion recording using a VG 70-250 SE (VG Analytical, UK) mass spectrometer (resolution 10,000) equipped with a HP 6890 gas chromatograph. Recoveries of individual internal standards were >60%. Limits of quantitation (LOQ) for PCDD/Fs, non-ortho PCBs, and other PCBs varied between 0.15-3.0, 1.0-5.0, 1.0-350, pg/g fat, respectively, depending on each individual congener. Concentrations were calculated with a lower bound method in which the results of congeners with concentrations below LOQ are designated as nil. The toxic equivalents (TEQ) were calculated with toxic equivalency factors (TEF) recommended by WHO in 1997 (van den Berg et al., 1998).

Quality control and assurance of the PCDD/F and PCB analyses; There was blind analysis and with laboratory and cross-sample contamination monitored by analysing procedural blank samples. During the study The Finnish Accreditation Service, FINAS, verified the competence of the laboratory (testing laboratory T077) in performing PCDD/F and PCB analyses in biological samples. The scope of accreditation includes PCDD/Fs, non-ortho PCBs, and other PCBs from biological samples. Placental concentration was used as a proxy for the milk concentration. The total PCDD/F and PCB exposure of each child was calculated from the concentration in the placental sample and the duration of breast-feeding (after having taken into account a yearly 25% first-order decline during lactation) [Alaluusua et al., 1996]. Placental exposure was estimated to correspond to the exposure via milk for two months [Alaluusua et al., 1999].

Dental examinations. Clinical examinations were performed at the mean age of 8.5 ± 0.45 years (range 7.2-10.1 years). The crowns of FPMs, mineralisation of which starts around birth and are completed during the first years of life, were

clinically examined for the presence and severity of MIH as previously described [Alaluusua et al., 1996]. The teeth were screened for demarcated opacity (mild defect), broken enamel (moderate defect) and loss of enamel with affected dentine, or atypical restoration replacing affected dental hard tissue (severe defect). Lesions smaller than 2 mm in diameter were not included. Children were categorised in four groups: a) those who had healthy molars, b) those who had molars with a mild defect, c) those who had molars with moderate defect or a mild lesion in more than one molar, and d) those who had molar(s) with severe defect(s). Dental examinations were carried out in the dental clinic by one dentist (SL) having no knowledge of the results of the PCDD/F or PCB analyses before the examination. During the same appointment, each mother was interviewed for the duration of breast-feeding of her child. Intra-examiner kappa coefficient for teeth with developmental defects of enamel was 0.91 and for classified defects (diffuse opacity, demarcated opacity and hypoplasia) 0.90. Corresponding inter-examiner kappa coefficients were 0.96 and 0.81.

Treatment of data. Difference in MIH occurrence between children with a cleft and those without was first compared by Pearson χ^2 test. The associations between the total exposure of PCDD/F or PCB and the presence and severity of MIH were evaluated by Pearson χ^2 test.

Results

A total of 24 of 167 children (14.4%) had MIH. Severe lesions were seen in 7 subjects and moderate defects or mild defects in more than one molar in 9 subjects. A mild lesion in only one molar was seen in 8 subjects. One of the 28 subjects with a cleft (3.6%) had MIH while 23 of 139 children without cleft (16.5%) had MIH. This difference was not statistically significant and therefore the subjects were further evaluated as one group.

The duration of breast-feeding ranged from 0 to 30 months (mean=7.2 ± 4.7), the median being 7 months (Table 1). $WHO_{PCDD/F}$TEQ ranged from 2.5 to 39.1 pg/g fat (mean=13.7 ± 6.8, median 12.4), and WHO_{PCB}TEQ from 0.7 to 9.8 pg/g fat (mean=2.7 ± 1.4, median 2.3). The mean sum of PCDD/Fs was 196 ± 105 pg/g fat and that of PCBs was 57.2 ± 28.1 ng/g fat. Both the calculated total exposure as expressed TEQs of PCDD/Fs and the total exposure of WHO_{PCB}TEQ were not associated with the occurrence or severity of MIH ($p > 0.05$). The duration of breast-feeding or the sum concentrations of PCDD/Fs or PCBs were also not associated with MIH or severity of MIH ($p > 0.05$).

Discussion

The present study shows that in a healthy Finnish child population born in late 1990's the exposure of a child to PCDD/Fs and PCBs calculated from the concentrations of these compounds in placenta and duration of breast-feeding was not associated with developmental enamel defects in the permanent first molars. In the earlier study on children born in 1987 an association was found between exposure to PCDD/Fs and dental defects [Alaluusua et al., 1996; Alaluusua et al., 1999] but the association between the defects and PCB exposure via mother's milk was weak. We assume that these previously controversial results concerning the consequences of PCDD/F exposure were a result of the decline in PCDD/F (and PCB) levels in Finland.

In our study of the children born in 1987 the sum of I-TEQs and PCB-TEQs in breast milk ranged from 3.8 pg/g to 99 pg/g fat and from 3.7 pg/g to 162 pg/g fat, respectively. In the present study the sum of $WHO_{PCDD/F}$TEQ ranged from 2.5 pg/g to 39.1 pg/g fat mean and was 70% of the concentration a decade before (Table 1). A three percent annual decline in PCDD/F-TEQ concentrations measured between the previous study on mother's milk in 1987 [Alaluusua et al., 1996] and the current study on placenta is in line with the trend data from mother's milk in Finland in general [Kiviranta, 2005]. Comparing WHO_{PCB}TEQ concentrations between mother's milk and placenta was not feasible as according to our experience PCBs have a greater tendency to accumulate in milk fat than in placental fat. The same phenomenon is seen with polybrominated diphenylethers when comparing concentrations of milk and placenta from the same mother [Main et al., 2007].

In addition to the decline in contaminant concentrations, the duration of breast feeding which was a contributory factor in the estimation of the total exposure to the toxic compounds, was shorter in the present study, the mean value being 7.2 months compared with that of 10.5 months in the earlier study (Table 1). However, in the present study, 14.4% of the children had MIH. The prevalence was almost as high as in children born in 1987, namely 17%. MIH is likely to be multifactorial and we assume that while the role of dioxins has decreased the role of other factors has increased. One possibility might be that the use of antibiotics in early childhood is one of those aetiological factors of MIH. A candidate antibiotic is amoxicillin that has been associated with enamel defects (fluorosis) in the FPMs in a recent prospective study [Hong et al., 2005]. Our own clinical and experimental observations support this finding [Laisi et al., unpublished data 2008]. The use of amoxicillin increased rapidly in the beginning of 1990's in Finland [Klaukka et al., 2006]. While in the 1980's its use was less than one daily dose/1,000 inhabitants/day, in mid 1990's the use had tripled. As amoxicillin is the first choice antibiotic for the treatment of common childhood diseases, such as the upper respiratory tract infections, it can be speculated that the increased use of amoxicillin might be associated with an increased prevalence of MIH.

Human milk concentrations of PCDD/Fs in the 1970's and 1980's reached the levels that were sufficient to cause dental defects in animal experiments. This could explain associations found in the past between dioxin exposure of a child via mother's milk and MIH. However, the levels of PCDD/Fs and

Table 1. Mean ±SD, and range of TCDD toxic equivalencies of PCDD/Fs and PCBs and duration of total breast-feeding in two different studies performed in approximately 10 years apart. In the first study [Alaluusua et al., 1996] human milk samples and in the second (present) study placental samples (used as a proxy for milk samples) were analysed.

	N	Parameter	Mean and SD	Range
Human milk samples collected in 1987	102	I-TEQ	19.8 ± 10.9 pg/g	3.8 – 99.4 pg/g
	102	PCB-TEQ	29.1 ± 20.4 pg/g	3.7 – 162 pg/g
	102	Duration of breast-feeding	10.5 ± 5.5 mo	1 – 36 mo
Placental samples collected in 1995-1999	167	$WHO_{PCDD/F}$TEQ	13.7 ± 6.8 pg/g	2.5 – 39.1 pg/g
	154	WHO_{PCB}TEQ	2.7 ± 1.4 pg/g	0.7 – 9.8 pg/g
	167	Duration of breast-feeding	7.2 ± 4.7 mo	0 – 30 mo

PCBs in breast milk in European countries have remarkably decreased since those years [Leeuwen and Malisch, 2002]. According to the present prospective study the exposure of children in 1995-1999 to PCDD/Fs and PCBs was clearly lower than in 1987 due to lower concentrations in milk/placenta and shorter breast-feeding periods. No statistically significant correlation between the exposure of the child to PCDD/Fs and PCBs via placenta/milk and MIH was found, suggesting that at the present background levels, these pollutants account for little or no dental aberrations in children. Even so, dioxins and PCBs are still one cause of developmental dental defects in highly polluted areas and in connection with dioxin accidents.

Conclusion

At prevailing levels, exposure of a child via placenta/mother's milk to PCDD/Fs and PCBs is not associated with MIH.

Acknowledgements

The study design was approved by the Ethics Committee of the National Public Health Institute, Finland. Informed consent was obtained from both the mother and her child. The study was supported by Academy of Finland (Contract 206689). For statistical support Mr. Jorma Torppa and for secretarial help Ms. Anneli Sinkkonen and Ms. Raila Jalomeri are acknowledged. The authors thank the volunteer mothers and their children and the contributions of research staffs in all maternity clinics and the laboratory of chemistry to the study.

References

Alaluusua S, Calderara P, Gerthoux PM, et al. Developmental dental aberrations after the dioxin accident in Seveso. Environ Health Perspect 2004:112:1313-1318.

Alaluusua S, Lukinmaa P-L. Developmental dental toxicity of dioxin and related compounds – a review. Int Dent J 2006;56:323-331.

Alaluusua S, Lukinmaa P-L, Torppa J, Tuomisto J, Vartiainen T. Developing teeth as biomarker of dioxin exposure. Lancet 1999:353:206.

Alaluusua S, Lukinmaa P-L, Vartiainen T, et al. Polychlorinated dibenzo-para-dioxins and dibenzofurans via mother's milk may cause developmental defects in the child's teeth. Environ Toxicol Pharmacol 1996;1:193-197.

Gao Y, Sahlberg C, Alaluusua S, et al. Lactational exposure of rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin impairs tooth biomineralisation. J Dent Res 2004;83:139-144.

Hong L, Levy SM, Warren JJ, et al. Association of amoxicillin use during early childhood with developmental tooth enamel defects. Arch Pediatr Adolesc Med 2005;139:943–948.

Hurst CH, DeVito MJ, Setzer RW. Acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: association of measured tissue concentrations with developmental effects. Toxicol Sci 2000;53:411-420.

Jan J, Sovcikova E, Ko an , Wsolova L, Trnovec T. Developmental dental defects in children exposed to PCBs in eastern Slovakia. Chemosphere 2007;67:S350-S354.

Kattainen H, Tuukkanen J, Simanainen U, et al. In utero/lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure impairs molar tooth development in rats. Toxicol Appl Pharmacol 2001 ;174:216-224.

Kiviranta H, Ovaskainen ML, Vartiainen T. Market basket study on dietary intake of OCDD/Fs, PCBs and PBDEs in Finland. Environ Int 2004;30:923-932.

Kiviranta H. Exposure and human PCDD/F and PCB body burden in Finland. Thesis, Publications of the National Public Health Institute, A 14/2005; 191. http://www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_a/2005/2005a14.pdf

Kiviranta H, Tuomisto JT, Tuomisto J, Tukiainen E, Vartiainen T. Polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in the general population in Finland. Chemosphere 2005:60:854-869.

Klaukka T, Helin-Salmivaara A, Huupponen R, Idänpään-Heikkilä J. Consumption of bacterium medicines in the downturn. Suomen Lääkärilehti 2006;61:27-31.

Leeuwen FXR van, Malisch R. Results of the third round of the WHO-coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. Organohalogen Compounds 2002:56;311-316.

Lukinmaa P-L, Sahlberg C, Leppäniemi A, Partanen A-M, Alaluusua S. Arrest of rat molar tooth development by lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin.Toxicol Appl Pharmacol 2001;173:38-47.

Main KM, Kiviranta H, Virtanen HE, et al. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environmental Health Perspectives 2007;115 (10): 1519-1526.

Partanen A-M, Kiukkonen A, Alaluusua S, et al. Developmental toxicity of dioxin to mouse embryonic teeth in vitro: arrest of tooth morphogenesis involves stimulation of apoptotic program in the dental epithelium. Toxicol Appl Pharmacol 2004;194:24-33.

U.S.A Environmental Protection Agency. In: http://www.epa.gov/ncea/pdfs/dioxin/dioxreass.htm.

Van den Berg M, Birnbaum L, Bosveld ATC, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106:775–792.

Vartiainen T, Saarikoski S, Jaakkola JJ, Tuomisto J. PCDD, PCDF, and PCB concentrations in human milk from two areas in Finland. Chemosphere 1997;34:2571-2583.

Wang SL, Chen TT, Hsu JF, et al. Neonatal and childhood teeth in relation to perinatal exposure to polychlorinated biphenyls and dibenzofurans: observations of the Yucheng children in Taiwan. Environ Res 2003;93:131-137.

Weerheijm KL, Jälevik B, Alaluusua S. Molar-Incisor Hypomineralisation (MIH). Caries Res 2001;35:390-391.