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Chapter 7

THE TOOTH, TARGET ORGAN OF BISPHENOL A, COULD BE USED AS A BIOMARKER OF EXPOSURE TO THIS AGENT

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ABSTRACT

Bisphenol A (BPA) is an endocrine disruptor chemical (EDC) whose effects on the development and function of reproductive organs and the mammary gland have been described for many years. In addition to these hormone-sensitive organs and cells, the tooth has been recently reported as an additional target organ of BPA [1]. The tooth comprises three compartments: the pulp cavity in its center, surrounded by dentin synthesized by odontoblasts, itself covered by enamel, the most mineralized tissue, synthesized by ameloblasts. After tooth eruption, ameloblasts disappear making any impact on enamel irreparable and thus irreversible. This property can be specifically exploited to relate the events that disturbed tooth development and especially the enamel mineralization taking place from the last third of fetal life to 4-5 years after birth in humans, precisely the time window of the highest susceptibility to EDCs. Experimental approaches consist to expose rats to low doses of BPA (alone or in combination with other enamel disrupting chemicals) and explore resulting enamel defects. Rats exposed to BPA present white opaque spots on their incisors reflecting enamel hypomineralization. These defects share many structural and biochemical characteristics with those described in the MIH (Molar Incisor Hypomineralization). MIH is an enamel disease that affects

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15-18 % of children aged 6 to 9 years, diagnosed by white to brown lesions on their permanent first molars and sometimes on their permanent incisors. These data may suggest that BPA could be a causative agent of MIH and therefore teeth affected by MIH could be used as early markers of exposure to BPA and / or agents acting similarly. Finally, these fragile and often decayed teeth lead the dentist to use filling materials which contain BPA monomers and can constitute an additional source of BPA contamination, at least locally. The potential BPA contamination and consequent adverse health effects resulting from the use of dental materials is an important issue actively discussed.

BPA, AN ACTIVE ENDOCRINE DISRUPTING CHEMICAL (EDC), IS ABLE TO IMPACT AMELOGENESIS

Oral Contamination of BPA

Bisphenol A (BPA), used in the manufacture of epoxy resins and polycarbonate plastics, is an exemplary widespread endocrine disruptor. BPA production has grown by 6 to 10% per year [2] and is now estimated at 4 million tons. Indeed, over 95% of populations examined, in all parts of the world, are contaminated, mainly *via* food and food packaging [3–6]. This contamination seems related to the population way of life since those limiting industrial goods, foods and habits are less contaminated [7]. 80% of whole BPA contamination result from oral exposure. Ingested BPA passes in blood circulation either by direct sublingual absorption [8] or by intestinal filtration. After hepatic detoxification and renal filtration, BPA-glucuronide is excreted in urine. Thus high urine and/or blood BPA concentrations may reflect high BPA exposure.

Epidemiological studies aim to associate high BPA urinary concentrations to adverse health effects including fertility troubles [9,10], obesity [11], diabetes [12–16], prostate cancer [17], increased risk of prematurity [18], and behavior problems [19–21]. Interestingly, BPA impact is not always evidenced on the whole population but more precisely on a selected group of individuals depending on age, sex, genetic background and the time of exposure.

Experimental Studies Carried out on Animals

Accordingly with epidemiological studies, experimental studies have shown that BPA impacts reproductive functions [22], prostate and mammary gland post-natal development [23,24]. It also targets adipocyte, hepatic and pancreatic β-cells explaining metabolic effects of BPA leading to obesity, diabetes and other metabolic defects [25–28]. An important point that has to be underlined is that most of these data provide evidence for the importance of the perinatal exposure and its consequence during adulthood, as well as the preferential impacts mainly recorded in males. The importance of BPA perinatal exposure has been illustrated in other studies on behavioral impact [29,30] and brain development [31].

Consistent with this, teeth have recently been found to be a target of BPA, where it disrupts amelogenesis leading to enamel hypomineralization [1].

How Can BPA Impact Amelogenesis?

The tooth comprises three compartments: the pulp cavity in its center, surrounded by dentin synthesized by odontoblasts, itself covered by enamel, the most mineralized tissue, synthesized by ameloblasts. Amelogenesis is a tightly time regulated process that includes ameloblast differentiation and enamel synthesis [32], and it is time-coordinated with odontogenesis.

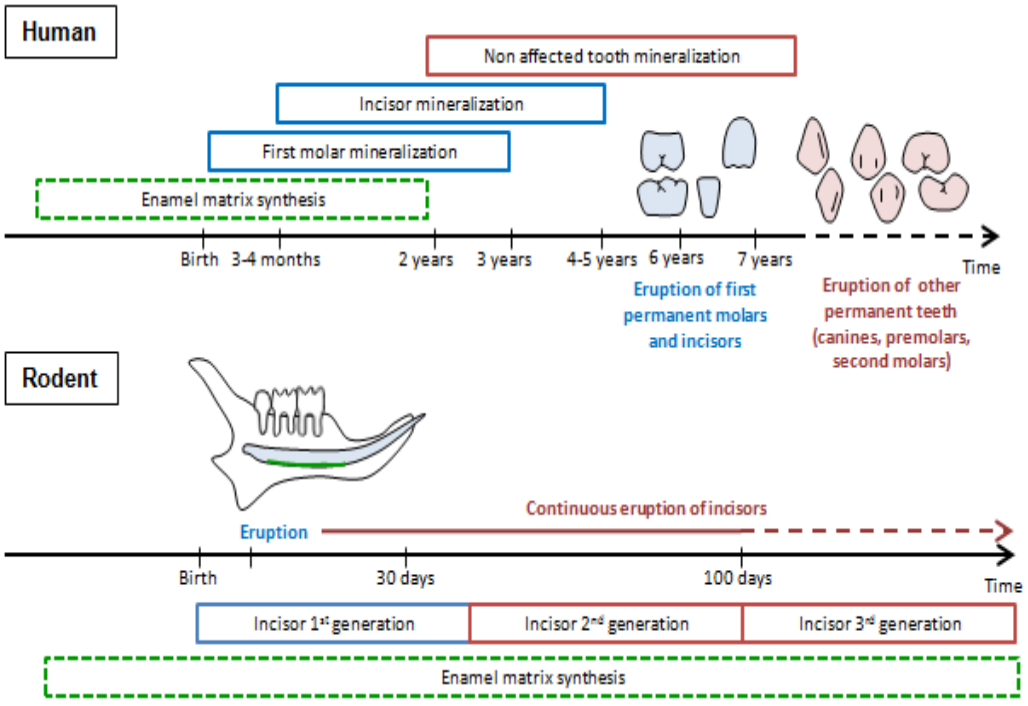

Figure 1. Chronology of tooth development in human and rodent. Amelogenesis begins first with enamel matrix synthesis mainly constituted of amelogenin, enamelin and ameloblastin during the secretion stage (in green). After that, matrix proteins are degraded by MMP20 and KLK4, and mineralization takes place. In blue, mineralization period of affected teeth by exposure to BPA and in red, mineralization period of non affected teeth.

Ameloblast differentiation originates in the dental cervical loop and begins with the secretory stage during which a partially mineralized enamel matrix is elaborated. The enamel matrix synthesized at that time is comprised of amelogenins, enamelin, ameloblastin and amelotin which are determinant for apatite deposition and for the first step of mineralization. When enamel mineralization is established and when crystal growth is determinant, matrix proteins are subject to extracellular processing by matrix metalloprotease-20 (MMP20). The matrix proteins are essential for the correct enamel formation as evidenced by the fact that mutations in any of the proteins involved leads to amelogenesis imperfecta (AI) [33,34]. Once the full thickness of the enamel has been deposited, amelogenesis enters the maturation phase during which the serine protease kallikrein 4 (KLK4) degrades the enamel matrix proteins and calcium actively deposited. Abnormal retention of proteins or the presence of any extraneous proteins such as serum albumin lead to the eruption of hypomineralized

enamel [35]. The rodent incisor provides a running record of how an impact on amelogenesis affects future development as the incisor continues to erupt [36,37] (figure 1). Once amelogenesis is complete, the ameloblasts and the overlying outer enamel epithelium cells degenerate and are ultimately lost through abrasion following tooth eruption. As a consequence, enamel defects are irreversible and provide a permanent record of any disturbances that occur during enamel development. This record of previous disturbances allows retrospective studies to be carried out and provides a means of temporally fixing a pathological event at some point during development. Among the different agents able to disturb amelogenesis, BPA has been recently shown to cause enamel hypomineralization [1].

Enamel Defects due to BPA Exposure Are Similar to MIH

BPA impact on amelogenesis was investigated by exposing Wistar rats daily by gavage to low doses of BPA (5 µg/kg/day) from conception until 30 or 100 days after birth [1]. At 30 days, 75% of male rats (and 30% of females) presented white opaque spots on their incisors. In complete contrast, incisors from BPA treated rats at 100 days were no longer affected and were indistinguishable from controls indicating that rat amelogenesis has a window of susceptibility that does not extend to the period when the incisor enamel in P100 rats was being laid down (figure 1). BPA affected enamel exhibited asymmetrical hypomineralization area similar to human MIH.

MIH is diagnosed in children at around 6 to 8 years of age and presents as random white opacities on the enamel of first permanent molars and sometimes on permanent incisors also [38,39]. Recent studies show that MIH prevalence turns around 15-18% of children affected whatever the region of the world considered (table 1) [40–57]. To date, MIH etiology remains unclear but the cause appears from environmental, even if enamelin gene polymorphism has been reported as a genetic factor associated with MIH development [52]. Given that MIH affects those teeth that are undergoing mineralization around the time of birth, it is clear that the enamel forming ameloblasts are only sensitive to the causative agent(s) responsible for MIH in a specific time window. MIH is indicative of some adverse event(s) occurring during early childhood that impact on enamel development [58]. Diverse environmental conditions such as medication (amoxicillin), hypoxia, hypocalcaemia, dioxins, polychlorinated biphenyls (PCBs) and prolonged breast feeding have been associated with MIH [59,60]. The white opaque opacities detected on 30 days-old rat incisors exposed to BPA provide a parallel between the etiology associated with the effect of BPA on rat amelogenesis and the etiology associated with human MIH. The perinatal window of sensitivity to BPA is precisely the same as the period of time corresponding to the mineralization of affected teeth, the first permanent molars and incisors (the first erupting permanent teeth). The mineralization of first permanent molars occurs between the 32th week of gestation and around four years after birth and that of permanent incisors between 3 months after birth and five years [61]. The mineralization of the other permanent teeth is delayed and they are very rarely affected by MIH.

Table 1. Prevalence of MIH since 2010. The prevalence doesn't seem to depend on particular geographical region. Mean prevalence recorded the last five years turns around 15%

Study	Number of children - Country	Age	MIH prevalence
da Costa-Silva CM, 2010	918 children - Brazil	6 to 12 years	19,8%
Brogårdh-Roth S et al., 2011	82 children - Sweden	10 to 12 years	16%
Biondi AM et al., 2011	1098 children - Buenos Aires Argentina	11,3 years	15,9%
Zawaideh FI et al., 2011	3666 children- Jordan	7 to 9 years	13,7%
Ghanim A et al., 2011	823 children - Irak	7 to 9 years	18,6%
Mahoney EK et al., 2011	235 children - Wellington, New Zealand	7 to 10 years	18,8%
Parikh DR et al., 2012	1366 children - India	8 to 12 years	9,2%
Balmer R et al., 2012	3233 children - North England	12 years	15,9%
Biondi AM et al., 2012	975 children - Argentina / Uruguay	11.6 years	6.56%
Ahmadi R et al., 2012	433 children - Iran	7-9 years	12.7%
Martínez Gómez TP et al., 2012	550 children - Spain	6 to 14 years	17,8%
Condò R et al., 2012	1500 children - Italy	4 to 15 years	7,3%
Jeremias F et al, 2013	1157 children - Brazil	6 to 12 years	12,3%
Grošelj M et al., 2013	558 children - Slovenia	6 to 11.5 years	21.4%
Garcia-Margarit M et al., 2014	840 children - Spain	8 years	21,8%
Mittal NP et al., 2014	1792 children - India	6 to 9 years	6,31%
Ng JJ et al., 2014	1083 children - Singapore	7.7 years	12.5%

Further investigations of both series of affected teeth by scanning electron microscopy showed that human MIH and BPA affected rat molars and incisors showed broken enamel in areas where the teeth occlude [1]. In addition, the prismatic structure in human MIH enamel as well as BPA exposed enamel (of 30 days old rats) was obscured by a covering organic layer similar to the one reported previously [62]. Among the main enamel matrix proteins (including amelogenins, enamelin and ameloblastin), enamelin expression (reported at the mRNA level as well as at the protein level) was higher in all BPA treated fractions. Enamelin amount is a central parameter for enamel synthesis as recently demonstrated by an experimental genetic approach [63]. Too much or too little enamelin abolishes the production of enamel crystals and prism structure. Thus, BPA is able to specifically target one key gene involved in amelogenesis and affect enamel. BPA has also been shown to decrease KLK4 expression which is involved in the degradation of enamel matrix proteins that can remain during the maturation process of enamel and inhibit normal apatite crystal growth. This second event strengthens the first one by increasing much more the amount of enamelin. In such case, extraneous proteins as serum albumin are able to accumulate in the poor quality enamel [64] reinforcing the hypomineralization finally diagnosed by the white opaque spots [65]. All these events were reproduced *in vitro* in the rat ameloblastic cell line HAT-7 showing the BPA transcriptional regulation of enamelin and KLK4 gene expression [1].

The mechanism by which BPA impacts on ameloblast gene expression is still unclear. However BPA has been shown to interact with multiple receptors [66] including steroid/retinoid/thyroid receptors [67,68]. BPA has been shown to bind estrogen receptors (ESRs) [69], GPR30 [70] ERRγ[71], but also retinoid receptor RORγ [72]. It modulates androgen, thyroid and glucocorticoid receptor activities and expression levels of key-transcription factors such as CREB, CEBP, STAT3, THR, PPARγ or GATA-4. BPA may have a direct influence on ameloblasts by binding to a BPA sensitive receptor. The presence of ESR alpha, vitamin D and thyroid receptors in ameloblasts [36,73] at the developmental stages where both enamelin and KLK4 expression is occurring maximally, invites the suggestion that BPA is affecting the expression of these genes through nuclear hormone pathway especially steroid receptors. The preferential enamel impact of BPA on male rats [74] supports this idea that should be further investigated.

Combinatorial Effects of BPA with Other EDCs and Enamel Hypomineralizing Factors

Human and animal populations are exposed to many EDCs simultaneously. BPA certainly acts in combination with other EDCs or hypomineralizing agents. These molecules do not necessarily share same structural properties, and act through different signaling pathways and receptors that have not been exhaustively characterized. Consequently, the effects of combinations of EDCs are unpredictable: they can potentiate each other's actions or, on the contrary, suppress them. Combination of low doses of BPA with low doses of genistein and vinclozolin, two other EDCs, didn't lead to a greater phenotype [75]. Whereas 6/8 rats exposed to BPA presented an enamel hypomineralization, only 3/8 presented similar white spots in the presence of combinations of BPA with either genistein or vinclozolin or both. Such results were explained by the differential modulation of enamelin and KLK4 expression by these three agents, only BPA is able to increase enamelin expression [75]. These results comfort the unpredictable impact of multiple agent combinations on a complex physiological function as amelogenesis.

In addition, one can suppose that BPA can have potentiated effects in the presence of hypomineralizing agents already described, especially dioxin that is also considered as a very active EDC [76].

Among the different exogenous agents able to affect enamel mineralization, fluoride present in drinking water causes dental fluorosis by targeting mature ameloblasts, inhibiting enzymatic activity and interacting with calcium in hydroxyapatite crystal [77,78]. The balance between beneficial effects on protection from caries and adverse effects leading to enamel hypomineralization occupies a narrow window of doses between 1 and 2 mg/day [79]. Other chemicals are also able to lead to enamel hypomineralization as lead that exacerbates fluorosis [80], tetracyclin and amoxicillin [81–83], and dioxin [76,84]. Interestingly, dioxin and amoxicillin exposures have been proposed as a causal factor of Molar Incisor Hypomineralisation (MIH) [85,86]. It is noteworthy that both factors increase enamel hypomineralization in the presence of fluoride [87,88] and the importance of the perinatal exposure to these agents has been underlined [59].

BPA MAY BE LEACHED FROM DENTAL RESINS

MIH affected teeth as well as any tooth with hypomineralized enamel (whatever the cause), are fragile and susceptible to caries. These decayed teeth lead the dentist to use filling materials which contain BPA monomers and can constitute an additional source of BPA contamination, at least locally. The issue concerning the evaluation of BPA contamination due to dental materials and their possible adverse health effects are hotly debated.

In Vitro Effects of BPA Contained in Dental Materials

Most dental sealants contain bisphenol-A diglycidyl methacrylate (bis-GMA), a common ingredient in restorative dental materials used since the 1960s. Olea and coworkers evidenced for the first time that dental resins leach BPA (present as an impurity) and related monomers bis-GMA, bis-DMA (bisphenol-A-diglycidyl-dimethacrylate) and BADGE (Bisphenol A diglycidyl ether) into saliva [89]. This paper relates the highest amount of BPA ever detected in saliva [90] but has the merit to raise the question of the safety around dental materials for patients but also for dentists. It triggered a lot of *in vitro* studies during the two last decades on potential effects of BPA (and related monomers) contained in dental resins, sealants, root canal sealers, orthodontic adhesives and polycarbonate brackets. These *in vitro* studies show that BPA leached from dental materials can present estrogen-like effects on hormone-sensitive cells, similar to other sources of BPA contaminations [89,91]. However, contrary to the first studies, the estrogen-like mitogenic activity of BPA related monomers, especially Bis-GMA, Bis-DMA and triethylene glycol dimethacrylate (TEGDMA) was not evidenced [92,93]. In addition, *in vitro* tests on Chinese hamster lung cells show that contrary to BPA, nor Bis-GMA nor Bis-DMA activate on CYP3A4 and CYP3A7 [94]. However, as BPA monomers can be digested by esterases present in the saliva giving rise to free BPA and as BPA can be leached from dental resins due to incomplete polymerization, their presence must be checked despite the lack of *in vitro* activity [95]. In addition, some dental materials are still able to released bis-GMA at least during one year after *in vitro* elution [96] raising the issue of long-term effects and contamination.

At low doses, BPA is able to induce cell proliferation by up-regulating hTERT expression [97] whereas at high doses it presents cytotoxic effects by reducing oral epithelial cell number [98]. It has also been shown that high-doses (µM to mM) of BPA monomers contained in dental resins present cytotoxic effects on gingival fibroblasts increasing cell apoptosis and DNA strand breakage [99,100]. In summary, these *in vitro* data showed that BPA contained in dental materials shares similar properties with those of other plastic containers and that oral cells are directly susceptible to BPA. The dose of exposure is an important parameter to take into account as BPA present opposite effects depending on the concentration, mitogenic effects at low doses and apoptotic or cytotoxic effects at high doses.

BPA Contamination due to Dental Resins and Composites

Nevertheless, these *in vitro* investigations do not answer to the important issue of the potential BPA contamination nor to any adverse health effects resulting from BPA containing dental materials that may occur after orthodontic and/or restorative treatments. That's why many epidemiological studies on the impact of dental resins and sealants were reported in the literature since the publication of Olea and coworkers in 1996 [89]. The consensus idea that emerges from the literature is that BPA concentrations in saliva and urine increased one to three hours after treatment then return to basal levels (table 2) [89,101–112], suggesting that sufficient gargling after treatment can remove BPA from the oral cavity [103]. Increased plasmatic levels of BPA were never reported whatever the study and the time of measurements after treatments.

However, some recently published papers related that the number of dental composites could lead to a significant long-term BPA contamination determined by salivary and urinary BPA concentrations [110–112]. The paper of Chung and co-workers presents the BPA urinary levels determined on 495 children aged 8-9 years and classified into four groups by the number of resin composites and sealant surfaces (0, 1-5, 6-10 and 11+). After adjusting for gender and age, the urinary BPA concentration in children with 11 or more resin composite surfaces was significantly higher than in the group without any resin composites. In the study of Han and co-workers, saliva of 124 children (62 controls with no dental sealant/resin and 62 cases with more than 4 tooth surfaces with dental sealant/resin) was analyzed.

The BPA level of control (0.40 µg/L) was significantly lower than that of case (0.92 µg/L). Kingman et al. [112] included 172 participants receiving composite restorations in their study and showed that BPA levels in urine may remain increased until 30 hours after treatment.

On the other hand, recent studies linking the number of dental resins composites and adverse health effects reported an increased level of anxiety, depression, social stress, interpersonal relation problems for children presenting more than two composites [107]. However, the number of composites is not associated to developmental troubles [108,109], nor to renal failure or damage [113,114] or immune function alterations [115]. These data point out the importance of the precise definition of the end-point and the difficulty to conclude of the non-effect of BPA in the absence of any positive control. They must be taken into account even if the number of subjects in cohorts is quite low to define further prospective studies including more BPA dosages during a longer period of time. Further studies are thus required before any conclusion on the possible BPA contamination by dental resins and possible adverse resulting effects.

Table 2. BPA concentrations in saliva and urine

Study	Number of subjects (age)	Time of measurements after treatments	Methods for BPA detection	Results	Conclusions
Olea N et al., 1996 [89]	18 subjects (18-25 years)	1 h	HPLC and MS	In saliva: - Baseline: no detection 1 h after treatment : 90-931 µg	The use of bis-GMA-based-resins in dentistry may represent an additional source of xenoestrogen exposure in humans.
Arenholt-Bindslev D et al., 1999 [101]	8 subjects (20-23 years)	Immediately, 1 h and 24 h	HPLC	In saliva: Baseline: no detection Immediately after treatment: 0.3-2.8 ppm 1 h and 24 h after treatment: no detection	Considerably lower amounts of BPA than previously reported, were detected in saliva samples collected immediately after but not 1 and 24 h after treatment.
Fung EY et al., 2000 (102)	40 subjects (20-55 years)	1 h, 3 h, 1 day, 3 and 5 days	HPLC	In saliva: 1 h and 3 h: 5.8-105.6 ppb in some specimens collected with a significant decrease from 1 to 3 h. In serum: - No detection beyond 3 h	BPA released orally from a dental sealant may not be absorbed or may be present in non detectable amounts in systemic circulation.
Sasaki N et al., 2005 [103]	21 subjects	Immediately	ELISA	In saliva: - 10 to 100 ng/ml of BPA	Sufficient gargling after treatment is important for risk management.
Joskow R et al., 2006 [104]	14 men	Immediately	MS	In saliva and urine: - 5.5 µg or 110 µg depending on the sealant used	Sealants should remain a useful part of routine preventive dental practice, especially those that leach negligible amounts of BPA.

Table 2. (Continued)

Study	Number of subjects (age)	Time of measurements after treatments	Methods for BPA detection	Results	Conclusions
Zimmerman-Downs JM et al., 2010 [105]	30 adults (18 - 40 years)	1 h, 3 h and 24 h	ELISA	In saliva: - 0.07 to 6.00 ng/ml at baseline Peak at 3 h: 3.98 ng/ml for one sealant group and 9.08 ng/ml for four sealant group 24 h: same levels as baseline. In serum: - No detection	Dental sealant material used in this study do not influence the serum concentration levels of BPA.
Kang YG et al., 2011 [106]	22 subjects	30 minutes, 1 day, 1 week and 1 month	HPLC / MS	In saliva: 20.889 ng/mL	The potential toxicity of BPA from placing lingual bonded retainer might be negligible.
Maserejian NN et al., 2012 [107]	534 children (6 - 10 years)	Comparison between the baseline and 5 years after treatments.	Psychosocial assessments by using validated instruments: Child Behavior Checklist (CBCL) parent-report, and Behavior Assessment for Children Self-Report (BASC-SR)		Greater exposure to bis-GMA based dental composite restorations was associated with impaired psychosocial function in children, whereas no adverse psychosocial outcomes were observed with greater UDMA-based compomer or amalgam treatment levels.

Study	Number of subjects (age)	Time of measurements after treatments	Methods for BPA detection	Results	Conclusions
Maserejian NN et al., 2012 [108]	534 children (6 - 10 years)	Comparison between the baseline and 5 years after treatments.	Tests of executive function, intelligence, memory, visual-spatial skills, verbal fluency, and problem-solving		Dental composite restorations had statistically insignificant associations of small magnitude with impairments in neuropsychological test change scores over 4- or 5-years of follow-up in this trial.
Maserejian NN et al., 2012 [109]	218 boys and 256 girls (6- 10 years)	Comparison between the baseline and 5 years after treatments	changes in BMI-for-age z-scores, body fat percentage (BF%), and height velocity; exploratory analyses (n = 113) examined age at menarche.		Children with more treatment on primary teeth had greater increases in BF% regardless of material type. Girls assigned to composites had lower risk of menarche during follow-up.
Chung SY et al., 2012 [110]	495 children (8-9 years)	Four groups based on the number of resin composites and sealant surfaces (0, 1-5, 6-10 and 11+)	HPLC-ESI-MS/MS	In urine: - 11 or more resin composite surfaces: 2.67 μg/g creatinine (creatinine-adjusted urinary BPA Concentrations)	Having many dental composite filling surfaces on teeth may increase the urinary BPA concentration in children.

Table 2. (Continued)

Study	Number of subjects (age)	Time of measurements after treatments	Methods for BPA detection	Results	Conclusions
Han DH et al., 2012 [111]	124 children	62 controls with no dental sealant/resin on their tooth surfaces and 62 cases with more than 4 tooth surfaces with dental sealant/resin	BPA ELISA Kit	In saliva: Control (0.40 μg/L) Case (0.92 μg/L)	There may be a relationship between salivary BPA level and dental sealant/resin.
Kingman A et al., 2012 [112]	172 subjects	Immediately, 30 h	LC/MS	In saliva: increase immediately (within 1 h) and return to prerestoration levels within 8 h after composite placement. In urine: return to prerestoration levels 9 to 30 h after restoration placement	Rubber dam use reduced BPA saliva concentrations but did not reduce the absorption of BPA (measured as BPA level in urine) during the study.

BPA Contaminations due to Orthodontic Materials

On the other hand, BPA and residual monomer leaching from orthodontic adhesive resins and polycarbonate brackets was evidenced *in vitro* as well as *in vivo* several months after treatment [116–118]. Even if their mitogenic activity was shown *in vitro* on the hormone-sensitive MCF-7 cell line [119], it is not possible to evaluate their *in vivo* potential adverse effect (if any) as there are no available data relating urinary or salivary BPA concentration measurements long-term after orthodontic treatments, nor any adverse health effect described in children receiving such treatment.

It is however interesting to note that the studies carried on animals did not show any toxic effects of BPA-GMA (contained in dental materials) on reproductive function [120].

In conclusion, at the present time, long-term BPA contamination by dental materials is actively discussed and controversial because of the small size of cohorts and the imprecise duration of exposure (table 2) [121]. Thus any related adverse effect is difficult, even impossible to establish. Further studies are required to answer to these important issues that accelerate active research in the field of dental materials in order to find inert materials either resistant to enzymatic, mechanic and chemical degradations or devoid of any BPA related monomers.

Taken all the published data together, the low dose effect (if any) of BPA leached by dental resins is probably negligible during adulthood. Nevertheless, more data are needed to evaluate the impact of orthodontic treatments often dispensed during childhood and the consequences on baby health of numerous resin composites carried by the mothers.

THE TEETH ARE PROPOSED AS EARLY BIOMARKER OF EXPOSURE TO BPA

Taking into account the huge amount of experimental data demonstrating BPA adverse impact on organ development and health, it is also important to lean on reliable biomarkers of exposure to BPA. The present data show that ameloblasts are susceptible to BPA and to other EDCs. Precise classification of the effects in each case would be valuable as these effects may not be as similar as may have been expected and specific enamel defects could reflect certain type of EDC combination. Considering the teeth as a complex organ resulting from epithelial and mesenchymal cell interactions, regrouping most of biological events and probably harboring many hormone receptors, the teeth or characterized *in vitro* models of enamel gene expression could constitute a novel marker of exposure to EDCs.

The data exposed in this paper underlie that BPA may be a causative agent in human MIH etiology. These fragile and often decayed teeth lead the dentist to use filling materials that may contain BPA based monomers thus maintaining pre-existing BPA contamination. MIH may represent a permanent record of exposure to BPA (or to EDCs sharing similar molecular effects) and could be easily used as a biomarker for retrospective analysis of infant exposure to EDCs and the impact of such exposure has on health in later life. It is interesting to note that many toxicants mentioned in enamel hypomineralization including BPA, dioxin and genistein, are also suspected to be involved in mammary cancer development [122] reinforcing the idea of common mechanisms in teeth and other organs.

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