



**HAL**  
open science

## **Prenatal exposure to persistent organic pollutants and molar-incisor hypomineralization among 12-year-old children in the French mother-child cohort PELAGIE**

Emile Boyer, Christine Monfort, Fabrice Lainé, Éric Gaudreau, Hélène Tillaut, Martine Bonnaure-Mallet, Sylvaine Cordier, Vincent Meuric, Cécile Chevrier

### ► To cite this version:

Emile Boyer, Christine Monfort, Fabrice Lainé, Éric Gaudreau, Hélène Tillaut, et al.. Prenatal exposure to persistent organic pollutants and molar-incisor hypomineralization among 12-year-old children in the French mother-child cohort PELAGIE. *Environmental Research*, 2023, 231, pp.116230. 10.1016/j.envres.2023.116230 . hal-04117438

**HAL Id: hal-04117438**

**<https://hal.science/hal-04117438>**

Submitted on 26 Jun 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Emile Boyer:** Conceptualization, Formal analysis, Investigation, Data curation, Writing Original draft, Writing Review and editing;

**Christine Monfort:** Methodology, Validation, Resources, Data curation, Writing Original draft, Writing Review and editing;

**Fabrice Lainé:** Investigation, Resources;

**Éric Gaudreau:** Investigation, , Data curation, Writing Original draft, Writing Review and editing;

**Hélène Tillaut:** Data curation, Writing Review and editing;

**Martine Bonnaure-Mallet:** Conceptualization, Investigation, Writing Review and editing;

**Sylvaine Cordier:** Conceptualization, Writing Review and editing, Project administration;

**Vincent Meuric:** Conceptualization, Investigation, Data curation, Writing Original draft, Writing Review and editing, Supervision;

**Cécile Chevrier:** Conceptualization, Formal analysis, Investigation, Writing Original draft, Writing Review and editing, Supervision, Project administration, Funding acquisition

## **Prenatal exposure to persistent organic pollutants and molar-incisor hypomineralization among 12-year-old children in the French mother-child cohort PELAGIE**

**Emile Boyer<sup>1\*</sup>, Christine Monfort<sup>2</sup>, Fabrice Lainé<sup>3</sup>, Éric Gaudreau<sup>4</sup>, Hélène Tillaut<sup>2</sup>, Martine Bonnaure-Mallet<sup>1</sup>, Sylvaine Cordier<sup>2</sup>, Vincent Meuric<sup>1</sup>, and Cécile Chevrier<sup>2</sup>**

<sup>1</sup>*INSERM, INRAE, Univ Rennes, CHU Rennes, Institut NUMECAN (Nutrition Metabolism and Cancer), F-35000 Rennes, France*

<sup>2</sup>*Univ Rennes, INSERM, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR\_S 1085, F-35000 Rennes, France*

<sup>3</sup>*INSERM, CIC 1414, Rennes, France*

<sup>4</sup>*Centre de Toxicologie du Québec (CTQ), Institut National de Santé Publique du Québec (INSPQ), Québec, Québec, Canada*

**\* Corresponding author**

Emile Boyer

16 rue du Maine 35137 BEDEE France

+33674758964

[emile.boyer@gmail.com](mailto:emile.boyer@gmail.com)

**Abstract**

**Background:** Exceptional episodes of exposure to high levels of persistent organic pollutants have already been associated with developmental defects of enamel among children, but knowledge is still scarce concerning the contribution of background levels of environmental contamination.

**Methods:** Children of the French PELAGIE mother-child cohort were followed from birth, with collection of medical data and cord blood samples that were used to measure polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), and perfluorinated alkyl substances (PFASs). At 12 years of age, molar-incisor hypomineralization (MIH) and other enamel defects (EDs) were recorded for 498 children. Associations were studied using logistic regression models adjusted for potential prenatal confounders.

**Results:** An increasing log-concentration of  $\beta$ -HCH was associated with a reduced risk of MIH and EDs (OR = 0.55; 95% CI, 0.32–0.95, and OR = 0.65; 95% CI, 0.43–0.98, respectively). Among girls, intermediate levels of p,p'-DDE were associated with a reduced risk of MIH. Among boys, we observed an increased risk of EDs in association with intermediate levels of PCB 138, PCB 153, PCB 187, and an increased risk of MIH with intermediate levels of PFOA and PFOS.

**Conclusions:** Two OCs were associated with a reduced risk of dental defects, whereas the associations between PCBs and PFASs and EDs or MIH were generally close to null or sex-specific, with an increased risk of dental defects in boys. These results suggest that POPs could impact amelogenesis. Replication of this study is required and the possible underlying mechanisms need to be explored.

**Keywords:** Environmental Pollution/adverse effects; Dental Enamel Hypoplasia; Molar Incisor Hypomineralization; Persistent Organic Pollutants; Polychlorinated Biphenyls; Organochlorine Compound; Per- and Polyfluoroalkyl Substances

**Sources of financial support**

The PELAGIE cohort has been funded by INSERM (since the beginning), the French Ministries of Health (2003-2004), Labor (2002-2003), and Research (ATC 2003-2004), the French National Institute for Public Health Surveillance (InVS, 2002-2006), the National Agency for Research (ANR, 2005-2008, 2010-2012, 2015-2019), the French Agency for Environmental Health Safety (Afsset/ANSES, 2007-2009, 2009-2012, 2019-2023), the French Agency for Drug Safety (2013-2017), the Fondation de France (2014-2017, 2015-2018, 2017-2020, 2019-2021, 2021-2024), the French Ministry of Ecology (PNRPE 2014-2016), the Research Institute of Public Health (IResP 2011-2014), and the following European programs: Hi-WATE (2007-2009), ENRIECO (2008-2010), OBERON (2019-2023), and REMEDIA (2020-2024).

## 1 Introduction

2  
3 Non-genetic dental enamel defects (EDs) appear as localized opacities, hypoplasia, or enamel  
4 breakdown and reflect disturbances that occurred during amelogenesis. Molar-incisor  
5 hypomineralization (MIH) refers to a distinct enamel condition, affecting at least one of the first  
6 permanent molars. Defined in 2001, this recently emerged clinical feature has since been a major  
7 topic of dental research<sup>1,2</sup>. Developmental incidents leading to EDs and MIH are highly prevalent  
8 (more than one in ten people), and severe cases are associated with increased dental needs,  
9 therefore constituting a public health problem<sup>3</sup>. Although many risk factors have been  
10 hypothesized, the etiology of these dental defects is still unclear. Given that MIH affect teeth that  
11 are undergoing mineralization around the time of birth, research is focused on this specific time  
12 window. Recent meta-analyses have proposed multifactorial models that include putative pre and  
13 perinatal factors, such as maternal illnesses, birth complications, and perinatal hypoxia<sup>2,4,5</sup>.  
14 Environmental pollution is also frequently cited as a potential risk factor<sup>5,6</sup>.

15 Human exposure to persistent organic pollutants (POPs), including polychlorinated biphenyls  
16 (PCBs), but also organochlorine pesticides (OCs), brominated flame retardants (BFRs), and  
17 perfluorinated alkyl substances (PFASs), is ubiquitous and concerns populations throughout the  
18 world<sup>7</sup>. POPs have been used in diverse activities from agriculture, vector control, and industrial  
19 production to building and material protection and transport. Due to their environmental  
20 persistence and bioaccumulative properties, the Stockholm Convention of Persistent Organic  
21 Pollutants was organized during the 1970s in order to prevent the production and discharge of  
22 POPs into the environment<sup>8</sup>. Most of these persistent compounds have thus been banned for  
23 several years. Their impact on human health at current environmental contamination levels is,  
24 however, still a matter of concern<sup>9</sup>. Detectable levels of OCs and PCBs are still found in a vast  
25 majority of pregnant women and mothers<sup>10,11</sup>. PFASs are still produced in large quantities  
26 worldwide (millions of tons annually) and have been detected in nearly 100% of biological samples  
27 of the recent European biomonitoring and epidemiological studies<sup>12,13</sup>.

28 Previous dental surveys reported dental abnormalities in the number (missing teeth and  
29 hypodontia), structure (chipped teeth and mottled enamel), and, to a lesser extent, shape related  
30 to early life exposure to dioxins and PCBs<sup>14</sup>. These findings have stemmed from observations made  
31 in the context of heavy exposure in Italy<sup>15</sup>, Japan<sup>16</sup>, Taiwan<sup>17</sup>, or in Slovenia and Slovakia<sup>18,19</sup>. A  
32 dose-response relationship between the level of exposure and percentage of affected teeth has  
33 also been reported in several studies<sup>14</sup>. Animal studies reported the possible role of dioxins on  
34 amelogenesis, suggesting the involvement of the aryl hydrocarbon receptor (AhR) pathway<sup>20</sup>.  
35 Another study reported an impaired dentinogenesis in rats treated with Hexachlorobenzene<sup>21</sup>.

36 Odontogenesis requires the fine-tuning of several growth factor signalling pathways<sup>22</sup> and there  
37 is increasing evidence of enamel synthesis alterations due to endocrine-disrupting chemicals<sup>23,24</sup>.  
38 On the other hand, information is still scarce concerning background levels of contamination in  
39 relation to MIH. As no data are currently available for other POPs than PCBs and dioxins with  
40 dental defects, we decided to extend this research hypothesis in this area.

41 Our aim was to explore the association between dental defects (EDs and MIH) and cord blood  
42 biomarkers of prenatal exposure to PCBs, OCs, and PFASs. This study was based on a prospective  
43 population-based mother-child cohort in the Brittany region that enabled us to collect cord blood  
44 samples at birth and perform complete dental examinations when the children reached 12 years  
45 of age.

**47 Methods****48 Study population**

49

50 The PELAGIE mother-child cohort included 3,421 pregnant women from the general population  
51 of the Brittany area (western France) at their first prenatal visit from 2002 to 2006. The women  
52 and their children were followed at birth with the collection of medical data and cord blood  
53 samples for approximately two thirds of the participants.

54 In total, 1,191 families were eligible for a clinical examination when their children were 12 years  
55 of age according to the following criteria: available prenatal cord blood samples (for POP chemical  
56 measurements), not lost to follow-up, and not wishing to quit the cohort. Among them, 258  
57 families (22%) could not be reached by telephone, 78 families (6.5%) chose to quit the cohort and  
58 296 families (25%) refused to participate or cancelled the appointment. Half of the refusals were  
59 due to the absence of oral consent from the 12-year-old children to undress for the Tanner stages  
60 or participate in the blood collection; other reasons were motivated by the parents due to  
61 logistical, time, and transport constraints, or were unknown due to no response after the first  
62 telephone contact.

63 In total, 559 children 12 years of age (60% of the families with telephone contact) gave consent  
64 to participate in the detailed clinical examination in the hospital setting between 2016 and 2018.  
65 The examination lasted 2.5 hours on average, and included metabolic, cardiovascular, and  
66 reproductive endpoints and an oral health examination, in addition to the assessment of pubertal  
67 stages and neurobehavioral endpoints.

68 At inclusion, women completed a self-administered questionnaire at home about their family,  
69 social, and demographic characteristics, diet, lifestyle, and the child's health.

70 The appropriate national ethics committees approved the study.

71

**72 Assessment of MIH and ED**

73

74 The oral health of the 12-year children was assessed by three senior pediatric dentists from the  
75 dental hospital using a standardized oral and periodontal examination protocol<sup>25</sup>. MIH-related  
76 hypomineralization was assessed for 498 children (89% of the participating subjects; dentists were  
77 not available for the remaining subjects) according to the diagnostic criteria of the European  
78 Academy of Paediatric Dentistry (EAPD; demarcated opacity, post-eruptive enamel breakdown,  
79 and atypical restoration or extraction due to MIH affecting at least one of the first permanent  
80 molars)<sup>26</sup>. Calibration was performed prior to the study on 30 patients with hypomineralization or  
81 enamel breakdown, and the inter-observer test yielded a kappa coefficient of 1 for diagnosis. In  
82 the case of healthy first permanent molars, teeth other than the first permanent molars with  
83 demarcated opacities were not diagnosed as being affected by MIH, but classified as EDs. The  
84 presence of demarcated opacities solely on maxillary incisors was considered to be due to trauma  
85 to the deciduous maxillary incisors. To avoid any bias, these teeth were not classified as EDs. Two  
86 variables were then designated: "ED", for patients with at least one enamel defect (with the  
87 limitation stated above); and "MIH", for patients with MIH with at least one first permanent molar  
88 with hypomineralization (according to the EAPD definition). The patients classified as MIH were  
89 therefore included among patients classified as ED.

90

**91 Cord blood POPs and PFASs measurements**

92

93 Cord blood samples were centrifuged immediately at the maternity hospital, transferred a few  
94 days later, and stored at  $-20^{\circ}\text{C}$  in our INSERM lab.

95 Levels of eight OCs [ $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH),  $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH),  
96 hexachlorobenzene (HCB), heptachlor epoxide (HCE), dieldrin,  $\alpha$ -endosulfan, p,p'-DDE, and p,p'-  
97 DDT), 14 PCBs (congeners 28, 52, 74, 99, 101, 118, 138, 153, 170, 180, 183, 187, 194, and 203),  
98 three polybrominated diphenyl ethers (PBDEs) (congeners 47, 99, and 209), and nine PFASs  
99 (perfluorooctanoic acid [PFOA], perfluorononanoic acid [PFNA], perfluorooctane sulfonate [PFOS],  
100 perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [PFHxS], perfluorodecanoic acid  
101 [PFDA], perfluorobutyric acid [PFBA], perfluorobutane sulfonate [PFBS], and perfluoroundecanoic  
102 acid [PFUDA]) were determined by the Centre de Toxicologie de Québec (CTQ), Institut national  
103 de santé publique du Québec (INSPQ) at two time periods (set 1:  $n = 155$  in 2008-2009 on a  
104 random subcohort<sup>27</sup> and set 2:  $n = 383$  in 2018-2019 on the rest of the cohort with a 12-year-old  
105 clinical follow-up; 21 participants had no cord blood sample available). The CTQ is a reference  
106 center in human toxicological analyses, complying with the current ISO standards (ISO/IEC 17025).  
107 It is also accredited for the organization of international inter-laboratory quality assurance  
108 programs. The processing of the serum samples and the extraction of the POP compounds (PCBs,  
109 OCs, and PBDEs) using gas chromatograph-mass spectrometry and PFASs using Ultra Performance  
110 Liquid Chromatography with tandem mass spectrometry are further described in supplementary  
111 content (Supplementary Data 1), as well as the measurement of the total lipid levels.

112 Among the children who had a dental examination at 12 years of age, PCBs and OCs were  
113 measured in 453 samples, while PFASs were measured in 404 samples (only in set 2018-2019). The  
114 reason for the missing values in these measurements was an insufficient volume of available cord  
115 serum. Due to analytical issues, there were additional missing values for PCB 138 and PFNA (11  
116 samples each).

117

## 118 **Statistical analysis**

119

120 POP measurements were considered in the present analyses when detection was  $> 70\%$ . Values  
121 below the limit of detection (LOD) were randomly imputed from a log-normal probability  
122 distribution, the parameters of which were estimated using a maximum-likelihood method<sup>28</sup>.  
123 Correlations between POPs concentration were computed using Pearson's coefficient. When  
124 missing data in the prenatal characteristics accounted for less than 5% of the subjects, values were  
125 imputed with the median and mode for quantitative and qualitative variables, respectively. This  
126 was the case for the following: pre-pregnancy BMI ( $n = 2$ ), maternal education ( $n = 1$ ), diabetes  
127 ( $n = 13$ ) and high blood pressure ( $n = 9$ ) before/during pregnancy, maternal smoking status at  
128 inclusion ( $n = 3$ ), and folic acid intake ( $n = 7$ ).

129 POP concentrations were first categorized in quartiles. The linearity of the relationship between  
130 these concentrations (in natural log-scale) and the odds of MIH and ED were assessed using  
131 restricted cubic splines based on full multivariate models<sup>29</sup>. We chose the 25th, 50th, and 75th  
132 percentiles as knots and rejected the assumption of linearity when the log-likelihood ratio test  
133 between the models, with and without restricted cubic splines, had a  $P$ -value  $< 0.05$ <sup>30</sup>.

134 For each POP, we performed multivariable logistic regression model to explore associations  
135 between cord blood POP concentrations in quartiles and the risk of MIH and ED. When linearity  
136 was not rejected, a second model that included continuous POP log-scale concentration was  
137 computed.

138 All models were *a priori* adjusted for maternal age at inclusion (quartiles), maternal smoking  
139 status at inclusion (nonsmoker/former smoker, smoker at conception, smoker) and maternal  
140 education (primary/secondary school, secondary school diploma, technical  
141 school/postsecondary). Additional adjustments were made for known or suspected risk factors  
142 and those that predicted outcomes or cord blood concentrations in this cohort ( $p < 0.20$ ): habitat  
143 (rural/semi-rural, urban), folic acid supplementation (dichotomous), and pre-pregnancy BMI  
144 (quartiles).

145 Analyses that took into account the total lipid concentration as an additional adjustment variable  
146 in the models for PCBs and OCs were performed as sensitivity analyses. In total, 42 subjects were  
147 excluded from these models due to aberrant values for the cord blood samples (i.e.,  $> 5$  g/L), which  
148 could be partially explained by possible faulty handling in the sample collection at birth.

149 Complementary analyses were carried out, such as stratifying by sex.

150

## 151 Results

### 152 Population

153

154 The prenatal characteristics of the subjects are summarized in Table 1. At enrolment, 18% of  
155 women lived in cities and most were postgraduates (72%). More than 13% reported smoking  
156 around the time of conception and having quit at the time of inclusion and 10% were still smoking  
157 at the time of inclusion. A few reported diabetes (3%) or high blood pressure (5%) before or during  
158 pregnancy and most had a pre-pregnancy BMI  $< 25$  kg/m<sup>2</sup> (86%). At birth, 49.8% of the children  
159 were girls. In total, 20% of children were diagnosed respectively with an ED and 9% with MIH.

160 EDs were more frequent for children whose mothers lived in rural/semi-rural areas during  
161 pregnancy (unadjusted OR = 1.6; 95% CI, 0.8–3.0). Equally, MIH was also more frequent for  
162 children of women living in rural/semi-rural areas (unadjusted OR = 5.5; 95% CI, 1.3–23.0), in  
163 addition to those who were smokers at inclusion (vs nonsmoker/ former smoker: unadjusted OR  
164 = 2.0; 95% CI, 0.8–4.4) or who had no folic acid supplementation (unadjusted OR = 2.4; 95% CI,  
165 0.9–6.1). MIH was also associated with lower maternal age at inclusion [vs fourth quartile:  
166 unadjusted OR (first quartile) = 2.50; 95% CI, 1.0–7.3, unadjusted OR (second quartile) = 2.32; 95%  
167 CI, 0.9–6.8, unadjusted OR (third quartile) = 2.52; 95% CI, 1.0–7.3]. Finally, MIH was associated with  
168 intermediate levels of pre-pregnancy BMI [vs fourth quartile: unadjusted OR (second quartile) =  
169 2.45; 95% CI, 1.0–6.6, unadjusted OR (third quartile) = 2.13; 95% CI, 0.9–5.8]. MIH frequency  
170 increased regularly with the cumulative number of these risk factors [group with one or zero risk  
171 factor: 0.0% (n = 31); group with two risk factors: 2.7% (n = 112); group with three risk factors:  
172 9.4% (n = 212); group with four or five risk factors: 17.0% (n = 143)].

173

### 174 Cord blood measurements

175

176 POPs retained in the analysis were those detected in more than 70% of the analyzed samples,  
177 which excluded all PBDEs, eight PCBs, five OCs, and five PFASs. Among the POPs retained, the  
178 detection rates ranged from 93 to 100%, except for PCB 187, for which a quarter of the subjects  
179 had values below the LOD (Table 2). The highest median concentrations were for PCB 153, p,p'-  
180 DDE, and PFOS. We observed high correlation coefficients within PCBs and OCs ( $0.6 < r < 0.9$ ) and  
181 within PFASs ( $0.3 < r < 0.7$ ), but low correlation coefficients between the three chemical families  
182 ( $r \approx 0.2$ ).

183

184 **Association between POPs and enamel defects**

185

186 The general pattern in the results of the multivariable logistic regression models showed a  
187 reduced or no risk for PCBs, p,p'-DDE, and HCB, without statistical significance (Table 3). The  
188 increasing log-concentration of  $\beta$ -HCH was significantly associated with a reduced risk of EDs and  
189 MIH (OR = 0.65; 95% CI, 0.43–0.98, and OR = 0.55; 95% CI, 0.32–0.95, respectively). The models  
190 showed similar results when adjusted for total lipid levels, except that the association between  $\beta$ -  
191 HCH and the risk of MIH became non-significant (see Supplementary Table 1). The data for 42  
192 subjects were excluded from these models due to aberrant values in total lipid concentration.

193 Among girls, there was a statistically significant association between moderate levels of p,p'-  
194 DDE and a decreased risk of MIH [vs first quartile: OR (second quartile) = 0.24; 95% CI, 0.06-0.86,  
195 OR (third quartile) = 0.08; 95% CI, 0.00-0.51, OR = 0.65 (fourth quartile); 95% CI, 0.19-2.19], but  
196 not for the risk of EDs (Table 3). Among boys, there were statistically significant associations  
197 between log-scaled  $\beta$ -HCH concentrations and a reduced risk of EDs (OR = 0.60; 95% CI, 0.35-0.99)  
198 and between intermediate levels of PCBs and an increased risk of EDs, without showing a linear  
199 dose-response relationship (second vs first quartile: OR = 2.96; 95% CI, 1.13-8.32 for PCB 138,  
200 OR = 3.24; 95% CI, 1.28-8.88 for PCB 153, and OR = 2.92; 95% CI, 1.15-7.86 for PCB 187).

201 For PFASs, the general pattern in the results among all children showed an increased or no risk  
202 for PFASs (Table 4). Among girls, there was no evidence of an association. Among boys, there was  
203 an increased risks of EDs for levels of PFOA above the median, with statistical significance for the  
204 third quartile (vs first quartile: OR = 2.97; 95% CI, 1.11-8.45) but not the fourth quartile (vs first  
205 quartile: OR = 2.14; 95% CI, 0.78-6.12). A statistically significant increased risk of MIH was  
206 observed with PFOS only for intermediate levels [vs first quartile: OR (second quartile) = 2.29; 95%  
207 CI, 0.38-19.1, OR (third quartile) = 6.73; 95% CI, 1.40-51.6 and OR (fourth quartile) = 1.76; 95% CI,  
208 0.30-14.1].

209

210 **Discussion**

211

212 In the present study, we observed a reduced risk of EDs and MIH among 12-year children  
213 associated with higher prenatal cord-concentrations of  $\beta$ -HCH, equally for both girls and boys. A  
214 reduced risk of MIH was also associated with moderate levels of p,p'-DDE among girls only. The  
215 association between cord-concentrations of PCBs and PFASs and the risk of EDs or MIH were  
216 generally close to null among all children. However, there may be sex-specific associations. Among  
217 boys only, there was an increased risk of EDs associated with intermediate prenatal levels of PCBs  
218 (in particular PCB 138, PCB 153, and PCB 187) and with the highest prenatal cord-concentrations  
219 of PFOA, as well as an increased risk of MIH with intermediate prenatal levels of PFOS.

220 In the present study, all subjects with at least one affected permanent tooth were classified in  
221 the ED group, regardless of the type of defect, and classified in the MIH group if at least one first  
222 permanent molar was affected, according to the diagnostic criteria<sup>31</sup>. Although the dental EDs  
223 include a wide range of symptoms, the examiners noted that they were predominantly  
224 demarcated opacities. White-spot lesions due to poor oral hygiene or dental fluorosis can  
225 sometimes lead to a misdiagnosis and overestimation of MIH. Nevertheless, children in the  
226 present study exhibited low dental plaque levels and caries experience. Systematic prescription of  
227 fluoride drops or tablets to all new-borns was encouraged in France in the 2000s<sup>32</sup>. However, the  
228 estimated prevalence of dental fluorosis was 0.5% in a population of children born in the same

229 years as children from the PELAGIE cohort<sup>33</sup>, and this kind of defects was not observed. Also, dental  
230 defects related to the use of specific antibiotics such as tetracyclines are not easily confused with  
231 MIH, as they imply very specific discolorations<sup>34</sup>. The prevalence of MIH found in our study (9%)  
232 was lower than the average calculated for Western Europe in the latest meta-analysis (15%)<sup>3</sup>.  
233 However, the authors reported large variations between studies, with the prevalence ranging from  
234 6% to 37%. Of note, no study reporting the prevalence of MIH in France was included in this meta-  
235 analysis, but a previous study in another French region reported a prevalence of 18%<sup>35</sup>.

236 The present study enrolled participants from the general population in the Brittany area in  
237 France and did not aim to achieve representativeness. Likely due to the voluntary participation,  
238 the population of the present study is more highly educated than expected in the source  
239 population. This should be considered in the interpretation of our results, including those of MIH  
240 prevalence. In addition, this implies that the results of the association analyses are unlikely to be  
241 confounded by large socioeconomic disparities. Moreover, we adjusted our association results for  
242 maternal educational level.

243 The literature investigating risk factors for EDs and or MIH has provided varying results and has  
244 not yet provided robust evidence. The role of various factors during the prenatal period has been  
245 suggested, such as maternal illness or psychological stress, which we did not measure in the  
246 present study<sup>4,5</sup>. We observed an increased risk of EDs among children for whom the mother lived  
247 in rural or semi-rural areas during pregnancy. Although access to healthcare is universal  
248 throughout France, there are still perinatal health inequalities in rural French regions, partially due  
249 to greater geographical remoteness and inadequate local healthcare<sup>36,37</sup>. We observed an  
250 increased risk of MIH for other prenatal factors, such as smoking status, the absence of folic acid  
251 intake, and younger maternal age. These results were, however, based on univariable models and  
252 should be therefore interpreted with caution. All these data were self-reports collected during  
253 pregnancy, and thus could not have been reported differentially according to the later diagnosis  
254 of ED or MIH. However, we cannot exclude a possible nondifferential reporting bias, such as  
255 underestimation of smoking during pregnancy, which would have prevented its observation as a  
256 risk factor in the present study. In any case, our association models were adjusted for these  
257 prenatal and study-specific factors when identified as potential confounders.

258 Relative to other European birth cohorts, the median cord-measurements of PCBs and OCs  
259 compounds observed in the PELAGIE cohort were among the highest for PCB 153 (PELAGIE = 0.120  
260 µg/L; range in European cohorts: 0.030-0.136 µg/L), in the middle of the range of the median  
261 values for p,p'-DDE (PELAGIE = 0.190 µg/L; range in European cohorts: 0.079-0.487 µg/L), and  
262 among the lowest for HCB (PELAGIE = 0.044 µg/l; range in European cohorts: 0.040-0.177 µg/l)<sup>11</sup>.  
263 The measurement of β-HCH in cord blood samples has been less frequent in the European studies,  
264 but the few existing studies reported similar or slightly lower concentrations than in the present  
265 study (0.051 µg/L or 12 ng/g lipid in PELAGIE vs 0.050 µg/L in Spain and 4.6 ng/g lipid in  
266 Belgium)<sup>38,39</sup>. Concerning the PFAS compounds measured in cord blood samples, the median  
267 values observed in the PELAGIE cohort were almost two-fold higher than those observed in birth  
268 cohorts of Belgium, the Netherlands<sup>11</sup>, Spain<sup>40</sup>, and the Faroes Islands<sup>41</sup>, ranging for PFOS from 1.6  
269 to 2.9 µg/L (vs 4.6 µg/L in PELAGIE) and for PFOA from 0.8 to 1.9 µg/L (vs 2.3 µg/L in PELAGIE).

270 While cord blood concentrations of OCs and PCBs appear to better reflect the exposure in late  
271 pregnancy<sup>42,43</sup>, there is no prior hypotheses of a specific prenatal window of vulnerability for EDs  
272 in the existing literature. Our main analyses included pollutant levels expressed on a wet-weight  
273 basis, as we assumed *a priori* no causal link between lipid content and EDs<sup>44</sup>. Models adjusted for  
274 total lipid levels provided comparable findings.

275 As stated above, the evidence of a role of PCBs contamination at background levels on EDs is  
276 limited. The previous epidemiological data concerned exceptional episodes of high exposure  
277 levels, and often implied a mixture of PCBs and dioxin compounds<sup>15-19</sup>. The results of the present  
278 study might appear to be concordant with those of these aforementioned studies. However, the  
279 associations we found concerning the OCs appear to contradict our initial study hypothesis. Some  
280 of the risk factors for ED and MIH identified in this study are well known to be related to OCs  
281 exposure levels. We observed a trend for decreased median levels of  $\beta$ -HCH (and p,p'-DDE) when  
282 accumulating the number of risk factors for MIH (Supplementary Table 2). However, this cannot  
283 be an explanation of the unexpected results of the association between  $\beta$ -HCH and MIH in the  
284 present study. Indeed, they were similar when the models were adjusted for this cumulative  
285 number of risk factors for MIH or when the analyses were restricted to this group of pregnant  
286 women accumulating these risk factors (Supplementary Table 3). Variations over time also did not  
287 explain this unexpected reduction in risk. Although we observed an increase in the prevalence of  
288 EDs and MIH with year of birth, the expected decrease in  $\beta$ -HCH exposure levels was barely  
289 observed in our data (Supplementary Table 4), and similar results were found with this additional  
290 adjustment (Supplementary Table 5). We ultimately have no explanation other than an  
291 unpredictable residual confounder or incidental finding that may have generated an artifact.

292 A recent review on acquired dental defects proposed the perturbation of the AhR nuclear  
293 translocator pathway as a common pathophysiological mechanism for xenobiotics to disrupt  
294 amelogenesis<sup>6</sup>. Authors also hypothesized that AhR pathway and metabolic balance of the calcium  
295 could be linked. The homeostasis of calcium metabolism is key in dental mineralization, with  
296 hypocalcaemia as a significant factor of enamel defect being reported for a long time in the  
297 literature<sup>45</sup>. While the action of dioxins and PCBs on AhR is established, its activation by  $\beta$ -HCH has  
298 only been demonstrated under *in vitro* conditions<sup>46</sup>. Although a protective effect has never been  
299 described, it is conceivable that  $\beta$ -HCH may induce other pathways that could act as  
300 compensators, or that the interactions, between pollutants, and with other etiological factors may  
301 modulate the toxicity.

302 Our study is the first to investigate the prenatal exposure to PFASs and tooth defects. Their  
303 potential impact on dental mineralized tissue is yet to be demonstrated, but have reportedly  
304 decreased bone density in humans<sup>47,48</sup>, and adverse skeletal deformities in animals<sup>49</sup> have already  
305 been observed in association with pre- and post-natal exposure to PFASs. On the other hand,  
306 exposure to PFASs has been shown to be associated with lower levels of thyroid hormone<sup>50</sup>.  
307 Thyroid hormone is thought to be involved in tooth development and maturation, and children  
308 with hypothyroidism have been reported to have enamel hypoplasia<sup>51</sup>. In-vitro and in-vivo studies  
309 have also suggested a negative effect of PFASs on insulin growth factor<sup>52</sup>, which is involved in the  
310 induction of enamel mineralization<sup>53</sup>. However, there is no reason for these mechanisms to be  
311 specific for boys. Another explanation could then involve the disruption of the reproductive  
312 endocrine. *In vivo*, PFOS has been shown to decrease expression of the Androgen Receptor in male  
313 rats<sup>54</sup>. *In vitro*, a dose-dependent inhibition of the Androgen Receptor nuclear translocation was  
314 observed for increasing concentrations of PFOA<sup>55</sup>. Interestingly, a study by Jedeon et al. showed  
315 that this receptor was also expressed in the rat ameloblasts, especially during the enamel  
316 mineralization phase (maturation stage), and that several genes involved in amelogenesis were  
317 regulated by Testosterone<sup>23</sup>.

318 As mentioned earlier, the origin of these defects in dental development is most likely  
319 multifactorial. Individual susceptibility may also be involved, as a genetic component for these  
320 defects has been recently highlighted<sup>56</sup>. POPs may be only one piece of the puzzle, but these  
321 chemicals should be considered for future studies on developmental defects of enamel.

322 Finally, it is worthwhile to note that we cannot rule out the possibility that some associations  
323 occurred by chance. We decided not to conduct statistical analyses for chemical mixtures, nor to  
324 correct for multiple comparisons. However, all statistical tests reported in the study were specified  
325 in advance (not post hoc) and all results are reported. The present study aimed at exploring  
326 question that has emerged from observational data after exceptional episodes of high exposure  
327 levels, with no clearly identified biological mechanisms involved, thus generating possible novel  
328 hypotheses, which will need to be confirmed by further experimental and observational studies.

329

### 330 **Conclusion**

331

332 Using cord-blood concentrations of POPs and dental examination at age 12 years of age, we  
333 found that prenatal exposure to  $\beta$ -HCH to be associated with a reduced risk of dental EDs and MIH.  
334 Among boys, intermediate levels of exposure to PCB 138, PCB 153, PCB 187, and the highest  
335 exposure levels of PFOS and PFOA were associated with an increased risk of these defects.  
336 Although a few studies have suggested a possible role of certain POPs after accidental episodes of  
337 high exposure levels on EDs in children, this is the first to explore the association with exposure at  
338 background levels of environmental contamination and the first to specifically examine PFASs.  
339 Further studies should be encouraged, as the present study does not exclude the prenatal role of  
340 POPs on amelogenesis.

**Table 1. Prenatal characteristics of the 12-year-old children examined for dental defects (n = 498; The mother-child PELAGIE cohort, France).**

Characteristic	Subcohort n = 498	ED n = 99	MIH n = 47
<b>Maternal age at inclusion (years)</b>			
[20.1, 27.9]	125 (25.1%)	28 (28.3%)	14 (29.8%)
(27.9, 30.5]	124 (24.9%)	29 (29.3%)	13 (27.7%)
(30.5, 33.3]	124 (24.9%)	23 (23.2%)	14 (29.8%)
(33.3, 43]	125 (25.1%)	19 (19.2%)	6 (12.8%)
<b>Pre-pregnancy BMI (kg/m<sup>2</sup>)</b>			
[16.2, 19.8]	125 (25.1%)	26 (26.3%)	10 (21.3%)
(19.8, 21.3]	125 (25.1%)	26 (26.3%)	16 (34.0%)
(21.3, 23.2]	124 (24.9%)	24 (24.2%)	14 (29.8%)
(23.2, 57.1]	124 (24.9%)	23 (23.2%)	7 (14.9%)
<b>Maternal education</b>			
Primary/Secondary school	53 (10.6%)	12 (12.1%)	5 (10.6%)
Secondary school diploma	85 (17.1%)	19 (19.2%)	12 (25.5%)
Technical school/Postsecondary	360 (72.3%)	68 (68.7%)	30 (63.8%)
<b>Habitat</b>			
Rural/semi-rural area (< 20,000 inhabitants)	408 (81.9%)	86 (86.9%)	45 (95.7%)
Urban (≥ 20,000 inhabitants)	90 (18.1%)	13 (13.1%)	2 (4.3%)
Diabetes before/during pregnancy	14 (2.8%)	1 (1.0%)	0 (0.0%)
High blood pressure before/during pregnancy	26 (5.2%)	7 (7.1%)	3 (6.4%)
<b>Maternal smoking status at inclusion</b>			
Non-smoker or former smoker	382 (76.7%)	78 (78.8%)	33 (70.2%)
Smoker at conception	65 (13.1%)	10 (10.1%)	6 (12.8%)
Smoker	51 (10.2%)	11 (11.1%)	8 (17.0%)
Fever during pregnancy (3 months before inclusion)	58 (11.6%)	9 (9.1%)	7 (14.9%)
Folic acid intake (3 months before inclusion)	104 (20.9%)	17 (17.2%)	5 (10.6%)
<b>Sex</b>			
Boys	250 (50.2%)	54 (54.5%)	23 (48.9%)
Girls	248 (49.8%)	45 (45.5%)	24 (51.1%)

Abbreviations: ED, enamel defect; MIH, molar-incisor hypomineralization.

**Table 2. Levels of persistent organic pollutants measured in cord blood samples (2003-2006; The mother-child PELAGIE cohort, France).**

Cord blood biomarkers	LOD( $\mu\text{g/L}$ )	$n$ (%) $\geq$ LOD	Cord blood concentration ( $\mu\text{g/L}$ )			
			p25	Median	p75	Max
PCB 118	0.01	449 (99.1%)	0.020	0.029	0.040	0.140
PCB 138 <sup>a</sup>	0.01	442 (100%)	0.048	0.069	0.096	0.500
PCB 153	0.01	453 (100%)	0.089	0.120	0.170	0.730
PCB 170	0.01	449 (99.1%)	0.022	0.031	0.043	0.240
PCB 180	0.01	453 (100%)	0.057	0.081	0.110	0.610
PCB 187	0.01	341 (75.3%)	0.010	0.014	0.020	0.200
p,p'-DDE	0.02	432 (95.4%)	0.120	0.190	0.330	3.300
$\beta$ -HCH	0.01	448 (99.1%)	0.036	0.051	0.075	1.300
Hexachlorobenzene	0.02	420 (92.7%)	0.033	0.044	0.057	0.250
PFHxS	0.06	404 (100%)	0.420	0.560	0.710	3.200
PFOS	0.40	404 (100%)	3.500	4.600	5.900	27.000
PFOA	0.07	404 (100%)	1.700	2.300	2.800	7.400
PFNA <sup>a</sup>	0.10	393 (100%)	0.300	0.310	0.450	1.500

Abbreviations: PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid. PCBs and OCs were measured in 453 samples. PFASs were measured in 404 samples. <sup>a</sup>11 samples with missing values due to isolated analytical issues.

**Table 3. Association of polychlorinated biphenyls and organochlorine pesticides levels with dental defect outcomes, and stratification by sex (The mother-child PELAGIE cohort, France).**

Persistent organic pollutant (µg/L)	N	ED				MIH			
		All		Girls	Boys	All		Girls	Boys
		N event	OR (95% CI)	OR (95% CI)	OR (95% CI)	N event	OR <sup>a</sup> (95% CI)	OR <sup>a</sup> (95% CI)	OR <sup>a</sup> (95% CI)
<b>PCB 118</b>									
≤ 0.020	116	29				18			
> 0.020 - 0.029	120	23	0.78 (0.40, 1.49)	0.59 (0.22, 1.55)	0.81 (0.32, 2.05)	11	0.65 (0.27, 1.51)	0.34 (0.08, 1.17)	1.00 (0.28, 3.58)
> 0.029 - 0.040	104	23	0.90 (0.46, 1.76)	0.79 (0.30, 2.06)	0.97 (0.38, 2.50)	10	0.69 (0.28, 1.67)	0.47 (0.12, 1.58)	0.66 (0.16, 2.59)
> 0.040	113	16	0.60 (0.29, 1.23)	0.68 (0.23, 1.95)	0.45 (0.16, 1.25)	6	0.40 (0.13, 1.09)	0.38 (0.07, 1.54)	0.34 (0.06, 1.53)
Log-concentration	453	91	0.83 (0.50, 1.37)	1.05 (0.48, 2.26)	– <sup>b</sup>	45	0.61 (0.30, 1.21)	0.60 (0.20, 1.70)	0.55 (0.20, 1.49)
<b>PCB 138</b>									
≤ 0.048	112	21				12			
> 0.048 - 0.069	109	26	1.52 (0.78, 3.01)	0.60 (0.21, 1.64)	2.96 (1.13, 8.32)	12	1.11 (0.45, 2.75)	0.44 (0.10, 1.60)	2.06 (0.52, 9.16)
> 0.069 - 0.096	112	21	1.23 (0.61, 2.50)	0.54 (0.17, 1.64)	2.41 (0.89, 6.92)	11	1.13 (0.45, 2.88)	0.44 (0.09, 1.80)	2.64 (0.64, 12.5)
> 0.096	109	19	1.26 (0.60, 2.65)	1.81 (0.64, 5.26)	0.69 (0.20, 2.28)	10	1.08 (0.40, 2.88)	1.44 (0.39, 5.29)	0.53 (0.09, 2.88)
Log-concentration	442	87	0.99 (0.59, 1.64)	1.25 (0.57, 2.82)	0.86 (0.43, 1.70)	45	0.77 (0.38, 1.52)	0.86 (0.30, 2.44)	– <sup>b</sup>
<b>PCB 153</b>									
≤ 0.089	116	25				16			
> 0.089 - 0.120	114	26	1.20 (0.63, 2.30)	0.60 (0.22, 1.61)	3.24 (1.28, 8.88)	11	0.75 (0.31, 1.76)	0.38 (0.09, 1.40)	1.56 (0.44, 5.69)
> 0.120 - 0.170	115	23	1.19 (0.60, 2.37)	1.11 (0.41, 2.99)	1.89 (0.67, 5.59)	9	0.64 (0.24, 1.61)	0.73 (0.19, 2.63)	0.63 (0.14, 2.67)
> 0.170	108	17	0.98 (0.45, 2.09)	1.39 (0.44, 4.34)	1.06 (0.34, 3.38)	9	0.73 (0.26, 1.95)	1.10 (0.25, 4.64)	0.59 (0.12, 2.70)
Log-concentration	453	91	0.91 (0.54, 1.56)	1.04 (0.45, 2.39)	0.88 (0.42, 1.81)	45	0.73 (0.35, 1.51)	0.83 (0.27, 2.51)	– <sup>b</sup>
<b>PCB 170</b>									
≤ 0.022	121	29				16			
> 0.022 - 0.031	111	18	0.69 (0.35, 1.36)	0.48 (0.15, 1.39)	1.32 (0.52, 3.38)	7	0.52 (0.18, 1.35)	0.51 (0.09, 2.20)	0.61 (0.14, 2.38)
> 0.031 - 0.043	113	26	1.23 (0.63, 2.41)	1.34 (0.51, 3.56)	1.64 (0.61, 4.50)	13	0.98 (0.40, 2.38)	1.44 (0.41, 5.14)	0.83 (0.20, 3.36)
> 0.043	108	18	0.95 (0.44, 2.04)	1.30 (0.43, 3.96)	0.73 (0.23, 2.24)	9	0.81 (0.28, 2.26)	1.26 (0.29, 5.45)	0.60 (0.12, 2.91)
Log-concentration	453	91	0.99 (0.58, 1.68)	1.28 (0.56, 2.95)	0.81 (0.39, 1.66)	45	0.81 (0.39, 1.67)	1.19 (0.40, 3.53)	0.69 (0.23, 1.91)
<b>PCB 180</b>									
≤ 0.057	117	26				15			
> 0.057 - 0.081	113	25	1.08 (0.56, 2.08)	0.94 (0.34, 2.56)	1.97 (0.80, 5.00)	9	0.71 (0.27, 1.77)	0.69 (0.15, 2.80)	1.41 (0.39, 5.18)
> 0.081 - 0.110	119	22	1.06 (0.52, 2.16)	1.12 (0.39, 3.23)	0.78 (0.27, 2.26)	12	0.94 (0.36, 2.41)	1.59 (0.42, 6.15)	0.56 (0.11, 2.62)
> 0.110	104	18	1.10 (0.51, 2.39)	1.94 (0.63, 6.10)	0.91 (0.28, 2.84)	9	0.97 (0.34, 2.74)	1.93 (0.43, 8.74)	0.82 (0.15, 4.15)

Log-concentration	453	91	0.85 (0.50, 1.45)	0.97 (0.43, 2.18)	– <sup>b</sup>	45	0.73 (0.35, 1.50)	0.97 (0.33, 2.84)	– <sup>b</sup>
PCB 187									
≤ 0.010	151	31				19			
> 0.010 - 0.014	77	20	1.63 (0.82, 3.22)	1.33 (0.49, 3.55)	2.92 (1.15, 7.86)	9	1.06 (0.41, 2.63)	0.66 (0.17, 2.36)	1.92 (0.53, 7.63)
> 0.014 - 0.020	114	24	1.22 (0.64, 2.31)	0.62 (0.20, 1.79)	2.40 (0.85, 7.11)	7	0.50 (0.18, 1.26)	0.27 (0.04, 1.24)	0.99 (0.20, 4.77)
> 0.020	111	16	0.94 (0.45, 1.93)	1.84 (0.63, 5.38)	0.76 (0.22, 2.50)	10	0.88 (0.34, 2.20)	1.57 (0.40, 5.93)	0.70 (0.13, 3.43)
Log-concentration	453	91	1.02 (0.65, 1.61)	1.41 (0.69, 2.93)	– <sup>b</sup>	45	0.85 (0.45, 1.58)	1.04 (0.39, 2.70)	0.75 (0.30, 1.80)
p,p'-DDE									
≤ 0.12	117	27				16			
> 0.12 - 0.19	120	24	0.87 (0.46, 1.64)	0.64 (0.24, 1.64)	1.29 (0.50, 3.36)	12	0.71 (0.30, 1.62)	0.24 (0.06, 0.86)	1.37 (0.37, 5.41)
> 0.19 - 0.33	111	16	0.62 (0.30, 1.22)	0.54 (0.17, 1.59)	0.88 (0.33, 2.32)	6	0.39 (0.13, 1.02)	0.08 (0.00, 0.51)	0.89 (0.22, 3.60)
> 0.33	105	24	1.23 (0.63, 2.39)	1.12 (0.41, 3.03)	1.29 (0.49, 3.41)	11	0.75 (0.31, 1.79)	0.65 (0.19, 2.19)	0.63 (0.13, 2.75)
Log-concentration	453	91	0.98 (0.74, 1.30)	1.01 (0.64, 1.61)	1.01 (0.70, 1.48)	45	0.80 (0.55, 1.17)	0.76 (0.41, 1.41)	0.77 (0.46, 1.35)
β-HCH									
≤ 0.036	120	31				18			
> 0.036 - 0.051	114	18	0.56 (0.28, 1.07)	0.44 (0.14, 1.23)	0.70 (0.27, 1.75)	9	0.50 (0.20, 1.17)	0.57 (0.13, 2.05)	0.66 (0.17, 2.36)
> 0.051 - 0.075	106	23	0.86 (0.45, 1.63)	0.78 (0.31, 1.93)	0.98 (0.39, 2.44)	9	0.54 (0.21, 1.30)	0.43 (0.10, 1.49)	0.65 (0.16, 2.39)
> 0.075	113	19	0.71 (0.35, 1.41)	0.91 (0.33, 2.46)	0.55 (0.20, 1.49)	9	0.53 (0.20, 1.29)	0.69 (0.18, 2.41)	0.51 (0.11, 2.08)
Log-concentration	453	91	0.65 (0.43, 0.98)	0.69 (0.35, 1.36)	0.60 (0.35, 0.99)	45	0.55 (0.32, 0.95)	0.50 (0.20, 1.24)	0.63 (0.30, 1.24)
Hexachlorobenzene									
≤ 0.033	122	24				13			
> 0.033 - 0.044	116	26	1.20 (0.63, 2.29)	1.11 (0.42, 2.89)	1.16 (0.47, 2.89)	13	1.03 (0.44, 2.43)	0.91 (0.25, 3.23)	1.05 (0.31, 3.72)
> 0.044 - 0.057	104	21	1.18 (0.60, 2.31)	1.42 (0.55, 3.72)	0.69 (0.25, 1.88)	12	1.21 (0.50, 2.90)	1.80 (0.55, 6.02)	0.62 (0.14, 2.55)
> 0.057	111	20	1.09 (0.54, 2.18)	1.05 (0.33, 3.17)	1.00 (0.39, 2.59)	7	0.72 (0.25, 1.94)	0.81 (0.14, 3.87)	0.62 (0.14, 2.54)
Log-concentration	453	91	0.98 (0.57, 1.70)	1.37 (0.58, 3.39)	0.77 (0.37, 1.59)	45	0.79 (0.38, 1.67)	1.26 (0.40, 4.29)	0.57 (0.19, 1.66)

Abbreviations: ED, enamel defect; MIH, molar-incisor hypomineralization.

All models were adjusted for maternal age (quartiles), pre-pregnancy BMI (quartiles), maternal smoking status at inclusion (non-smoker/former smoker, smoker at conception, smoker), maternal education (primary/secondary school, secondary school diploma, technical school/postsecondary), and habitat (rural/semi-rural, urban).

<sup>a</sup>Additional adjustment made for folic acid intake (dichotomous). <sup>b</sup>The assumption of linearity was rejected.

**Table 4. Association of perfluorinated alkyl substances levels with dental defect outcomes, and stratification by sex (The mother-child PELAGIE cohort, France).**

Persistent organic pollutant (µg/L)	N	ED			MIH				
		All		Girls	Boys	All		Girls	Boys
		N event	OR (95% CI)	OR (95% CI)	OR (95% CI)	N event	OR <sup>a</sup> (95% CI)	OR <sup>a</sup> (95% CI)	OR <sup>a</sup> (95% CI)
<b>PFHxS</b>									
≤ 0.42	103	18				11			
> 0.42 - 0.56	101	25	1.68 (0.83, 3.43)	1.44 (0.48, 4.37)	1.85 (0.71, 4.99)	13	1.37 (0.55, 3.47)	1.13 (0.30, 4.31)	1.35 (0.34, 5.55)
> 0.56 - 0.71	100	21	1.37 (0.67, 2.84)	2.00 (0.68, 6.13)	1.01 (0.37, 2.79)	10	1.17 (0.44, 3.08)	1.28 (0.33, 4.94)	0.91 (0.22, 3.79)
> 0.71	100	18	1.04 (0.49, 2.19)	0.76 (0.22, 2.51)	1.17 (0.43, 3.20)	7	0.61 (0.21, 1.69)	0.46 (0.09, 2.04)	0.50 (0.09, 2.33)
Log-concentration	404	82	– <sup>b</sup>	1.53 (0.65, 3.86)	1.16 (0.57, 2.39)	41	1.01 (0.49, 2.14)	1.29 (0.47, 3.93)	0.73 (0.24, 2.22)
<b>PFOS</b>									
≤ 3.5	103	19				8			
> 3.5 - 4.6	101	25	1.62 (0.81, 3.30)	2.62 (0.91, 8.01)	1.27 (0.47, 3.46)	12	1.77 (0.66, 4.96)	2.24 (0.64, 8.47)	2.29 (0.38, 19.1)
> 4.6 - 5.9	100	18	1.03 (0.49, 2.16)	1.27 (0.42, 3.92)	1.26 (0.47, 3.46)	11	1.57 (0.57, 4.45)	1.06 (0.26, 4.28)	6.73 (1.40, 51.6)
> 5.9	100	20	1.13 (0.55, 2.33)	1.09 (0.31, 3.68)	1.12 (0.40, 3.12)	10	1.42 (0.51, 4.04)	1.02 (0.19, 4.85)	1.76 (0.30, 14.1)
Log-concentration	404	82	0.97 (0.56, 1.71)	1.08 (0.46, 2.67)	1.04 (0.49, 2.25)	41	1.08 (0.52, 2.28)	1.03 (0.35, 3.28)	– <sup>b</sup>
<b>PFOA</b>									
≤ 1.7	107	20				12			
> 1.7 - 2.3	114	19	0.83 (0.40, 1.68)	1.21 (0.43, 3.41)	0.69 (0.22, 2.07)	9	0.60 (0.22, 1.55)	0.80 (0.21, 2.87)	0.65 (0.11, 3.47)
> 2.3 - 2.8	85	21	1.54 (0.75, 3.18)	0.63 (0.20, 1.90)	2.97 (1.11, 8.45)	8	0.86 (0.31, 2.30)	0.36 (0.08, 1.48)	1.89 (0.39, 10.0)
> 2.8	98	22	1.32 (0.66, 2.68)	0.85 (0.26, 2.65)	2.14 (0.78, 6.12)	12	1.28 (0.52, 3.17)	0.82 (0.18, 3.31)	2.66 (0.63, 13.2)
Log-concentration	404	82	1.41 (0.76, 2.66)	0.97 (0.35, 2.68)	2.16 (0.93, 5.41)	41	1.26 (0.54, 3.03)	0.65 (0.18, 2.40)	2.99 (0.76, 13.6)
<b>PFNA</b>									
≤ 0.29	96	18				9			
> 0.29 - 0.31	101	21	1.35 (0.65, 2.83)	0.90 (0.28, 2.90)	1.77 (0.65, 4.99)	8	0.95 (0.32, 2.75)	1.35 (0.29, 6.66)	0.67 (0.12, 3.28)
> 0.31 - 0.45	101	21	1.39 (0.66, 2.93)	1.38 (0.47, 4.23)	1.65 (0.58, 4.81)	12	1.57 (0.59, 4.36)	2.19 (0.53, 10.3)	1.59 (0.39, 7.06)
> 0.45	95	22	1.48 (0.72, 3.08)	0.79 (0.25, 2.47)	1.63 (0.55, 4.93)	12	1.68 (0.64, 4.57)	1.32 (0.30, 6.24)	1.17 (0.23, 5.79)
Log-concentration	393	82	1.23 (0.76, 2.02)	0.90 (0.47, 1.80)	1.76 (0.81, 4.05)	41	1.63 (0.84, 3.38)	1.58 (0.65, 4.52)	1.94 (0.60, 7.07)

Abbreviations: ED, enamel defect; MIH, molar-incisor hypomineralization; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid.

All models were adjusted for maternal age (quartiles), pre-pregnancy BMI (quartiles), maternal smoking status at inclusion (non-smoker/ former smoker, smoker at conception, smoker), maternal education (primary/secondary school, secondary school diploma, technical school/postsecondary), and habitat (rural/semi-rural, urban).

<sup>a</sup>Additional adjustment made for folic acid intake (dichotomous). <sup>b</sup>The assumption of linearity was rejected.

**Acknowledgments**

We are grateful to the physicians and nurses and all the families who participated and continue to participate in the study. We specially thank the UIC health personnel, Stéphanie Métayé, Cécile Réminiac and Alexia Marie-Cousin for their rigorous work in examining the children, and Ronan Garlantézec, Charline Warembourg, Nathalie Costet, Florence Rouget, Véronique Villalon, and Isabelle Dorval who contributed to the PELAGIE cohort in general.

Journal Pre-proof

1. Weerheijm KL, Jälevik B, Alaluusua S. Molar-incisor hypomineralisation. *Caries Res.* 2001;35(5):390-391. doi:10.1159/000047479
2. Rodd HD, Graham A, Tajmehr N, Timms L, Hasmun N. Molar incisor hypomineralisation: current knowledge and practice. *Int Dent J.* 2020;71(4):285-291. doi:10.1111/idj.12624
3. Schwendicke F, Elhennawy K, Reda S, Bekes K, Manton DJ, Krois J. Global burden of molar incisor hypomineralization. *J Dent.* 2018;68:10-18. doi:10.1016/j.jdent.2017.12.002
4. Garot E, Rouas P, Somani C, Taylor GD, Wong F, Lygidakis NA. An update of the aetiological factors involved in molar incisor hypomineralisation (MIH): a systematic review and meta-analysis. *Eur Arch Paediatr Dent.* Published online June 24, 2021. doi:10.1007/s40368-021-00646-x
5. Fatturi AL, Wambier LM, Chibinski AC, et al. A systematic review and meta-analysis of systemic exposure associated with molar incisor hypomineralization. *Community Dent Oral Epidemiol.* 2019;47(5):407-415. doi:10.1111/cdoe.12467
6. Collignon AM, Vergnes JN, Germa A, et al. Factors and Mechanisms Involved in Acquired Developmental Defects of Enamel: A Scoping Review. *Front Pediatr.* 2022;10:836708. doi:10.3389/fped.2022.836708
7. WHO UNEP. *State of the Science of Endocrine Disrupting Chemicals 2012.* (Heindel JJ, Jobling S, Kidd K, Zoeller TR, eds.). World Health Organization; 2013. Accessed November 15, 2021. <https://apps.who.int/iris/handle/10665/78101>
8. Fiedler H. National PCDD/PCDF release inventories under the Stockholm Convention on Persistent Organic Pollutants. *Chemosphere.* 2007;67(9):S96-108. doi:10.1016/j.chemosphere.2006.05.093
9. Demeneix B, Slama R. *Endocrine Disruptors: From Scientific Evidence to Human Health Protection Policy.* Publications Office of the European Union; 2019. Accessed November 8, 2021. <https://data.europa.eu/doi/10.2861/802173>
10. Govarts E, Nieuwenhuijsen M, Schoeters G, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. *Environ Health Perspect.* 2012;120(2):162-170. doi:10.1289/ehp.1103767
11. Govarts E, Iszatt N, Trnovec T, et al. Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: Pooled analysis of seven European birth cohorts. *Environ Int.* 2018;115:267-278. doi:10.1016/j.envint.2018.03.017
12. Apel P, Angerer J, Wilhelm M, Kolossa-Gehring M. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. *Int J Hyg Environ Health.* 2017;220(2 Pt A):152-166. doi:10.1016/j.ijheh.2016.09.007
13. Casas M, Chevrier C, Hond ED, et al. Exposure to brominated flame retardants, perfluorinated compounds, phthalates and phenols in European birth cohorts: ENRIECO evaluation, first human biomonitoring results, and recommendations. *Int J Hyg Environ Health.* 2013;216(3):230-242. doi:10.1016/j.ijheh.2012.05.009
14. Alaluusua S, Lukinmaa PL. Developmental dental toxicity of dioxin and related compounds--a review. *Int Dent J.* 2006;56(6):323-331. doi:10.1111/j.1875-595x.2006.tb00336.x
15. Alaluusua S, Calderara P, Gerthoux PM, et al. Developmental dental aberrations after the dioxin accident in Seveso. *Environ Health Perspect.* 2004;112(13):1313-1318. doi:10.1289/ehp.6920
16. Masuda Y. The Yusho Rice Oil Poisoning Incident. In: Schecter A, ed. *Dioxins and Health.* Springer US; 1994:633-659. doi:10.1007/978-1-4899-1462-0\_19
17. 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(2):131-137. doi:10.1016/s0013-9351(03)00040-9
18. Jan J, Vrbic V. Polychlorinated biphenyls cause developmental enamel defects in children. *Caries Res.* 2000;34(6):469-473. doi:10.1159/000016625
19. Jan J, Sovcikova E, Kocan A, Wsolova L, Trnovec T. Developmental dental defects in children exposed to PCBs in eastern Slovakia. *Chemosphere.* 2007;67(9):S350-354. doi:10.1016/j.chemosphere.2006.05.148

20. Bianchi S, Bernardi S, Benini M, Valvona G, Macchiarelli G. Exposure to persistent organic pollutants during tooth formation: molecular mechanisms and clinical findings. *Rev Environ Health*. 2020;35(4):303-310. doi:10.1515/reveh-2019-0093
21. Long PH, Herbert RA, Nyska A. Hexachlorobenzene-induced incisor degeneration in Sprague-Dawley rats. *Toxicol Pathol*. 2004;32(1):35-40. doi:10.1080/01926230490260871
22. Wang XP, Thesleff I. Tooth Development. In: Unsicker K, Krieglstein K, eds. *Cell Signaling and Growth Factors in Development*. John Wiley & Sons, Ltd; 2005:719-754. doi:10.1002/9783527619689.ch19
23. Jedeon K, Loiodice S, Salhi K, et al. Androgen Receptor Involvement in Rat Amelogenesis: An Additional Way for Endocrine-Disrupting Chemicals to Affect Enamel Synthesis. *Endocrinology*. 2016;157(11):4287-4296. doi:10.1210/en.2016-1342
24. Jedeon K, Loiodice S, Marciano C, et al. Estrogen and bisphenol A affect male rat enamel formation and promote ameloblast proliferation. *Endocrinology*. 2014;155(9):3365-3375. doi:10.1210/en.2013-2161
25. World Health Organization. *Oral Health Surveys: Basic Methods*. World Health Organization; 2013.
26. Weerheijm KL, Duggal M, Mejère I, et al. Judgement criteria for molar incisor hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003. *Eur J Paediatr Dent*. 2003;4(3):110-113.
27. Chevrier C, Warembourg C, Gaudreau E, et al. Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. *Epidemiology*. 2013;24(2):251-260. doi:10.1097/EDE.0b013e31827f53ec
28. Jin Y, Hein MJ, Deddens JA, Hines CJ. Analysis of lognormally distributed exposure data with repeated measures and values below the limit of detection using SAS. *Ann Occup Hyg*. 2011;55(1):97-112. doi:10.1093/annhyg/meq061
29. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med*. 2010;29(9):1037-1057. doi:10.1002/sim.3841
30. Meng XL, Rubin DB. Performing likelihood ratio tests with multiply-imputed data sets. *Biometrika*. 1992;79(1):103-111. doi:10.1093/biomet/79.1.103
31. Ghanim A, Elfrink M, Weerheijm K, Mariño R, Manton D. A practical method for use in epidemiological studies on enamel hypomineralisation. *Eur Arch Paediatr Dent*. 2015;16(3):235-246. doi:10.1007/s40368-015-0178-8
32. Bocquet A, Bresson JL, Briend A, et al. [Fluoride supplementation must be initiated at birth in children in France]. *Arch Pediatr*. 2002;9(11):1211-1212. doi:10.1016/s0929-693x(02)00100-8
33. Chevallier C, Harel S. *Study of the oral health of children from "CP" and "CM2" classes in Rennes state-funded schools in 2007*. Thèse d'exercice. Université de Rennes 1; 2008. Accessed April 1, 2022. <https://www.sudoc.fr/123494567>
34. Tredwin CJ, Scully C, Bagan-Sebastian JV. Drug-induced disorders of teeth. *J Dent Res*. 2005;84(7):596-602. doi:10.1177/154405910508400703
35. Fahd CD. *Prevalence of molar-incisor hypomineralisation among a group of children from the south of France*. Bordeaux; 2018. Accessed April 1, 2022. <https://dumas.ccsd.cnrs.fr/dumas-01934899>
36. Cabailot A, Lavarenne M, Vaure Chiffre J, et al. Perceptions and behaviour of pregnant women in socioeconomic deprivation in rural areas. A qualitative study in France. *Health Expect*. Published online June 15, 2022. doi:10.1111/hex.13472
37. Combier E, Charreire H, Le Vaillant M, et al. Perinatal health inequalities and accessibility of maternity services in a rural French region: closing maternity units in Burgundy. *Health Place*. 2013;24:225-233. doi:10.1016/j.healthplace.2013.09.006
38. Vizcaino E, Grimalt JO, Lopez-Espinosa MJ, Llop S, Rebagliato M, Ballester F. Maternal origin and other determinants of cord serum organochlorine compound concentrations in infants from the general population. *Environ Sci Technol*. 2010;44(16):6488-6495. doi:10.1021/es101397e
39. Vriens A, Nawrot TS, Baeyens W, et al. Neonatal exposure to environmental pollutants and placental mitochondrial DNA content: A multi-pollutant approach. *Environ Int*. 2017;106:60-68. doi:10.1016/j.envint.2017.05.022

40. Manzano-Salgado CB, Casas M, López-Espinosa MJ, et al. Transfer of perfluoroalkyl substances from mother to fetus in a Spanish birth cohort. *Environ Res.* 2015;142:471-478. doi:10.1016/j.envres.2015.07.020
41. Blomberg AJ, Shih YH, Messerlian C, Jørgensen LH, Weihe P, Grandjean P. Early-life associations between per- and polyfluoroalkyl substances and serum lipids in a longitudinal birth cohort. *Environ Res.* 2021;200:111400. doi:10.1016/j.envres.2021.111400
42. Junqué E, Garcia S, Martínez MÁ, Rovira J, Schuhmacher M, Grimalt JO. Changes of organochlorine compound concentrations in maternal serum during pregnancy and comparison to serum cord blood composition. *Environ Res.* 2020;182:108994. doi:10.1016/j.envres.2019.108994
43. Vizcaino E, Grimalt JO, Fernández-Somoano A, Tardon A. Transport of persistent organic pollutants across the human placenta. *Environ Int.* 2014;65:107-115. doi:10.1016/j.envint.2014.01.004
44. Schisterman EF, Whitcomb BW, Louis GMB, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environ Health Perspect.* 2005;113(7):853-857. doi:10.1289/ehp.7640
45. Nikiforuk G, Fraser D. Chemical determinants of enamel hypoplasia in children with disorders of calcium and phosphate homeostasis. *J Dent Res.* 1979;58(Spec Issue B):1014-1015. doi:10.1177/002203457905800208011
46. Rubini E, Paglia G, Cannella D, et al.  $\beta$ -Hexachlorocyclohexane: A Small Molecule with a Big Impact on Human Cellular Biochemistry. *Biomedicines.* 2020;8(11):E505. doi:10.3390/biomedicines8110505
47. Khalil N, Chen A, Lee M, et al. Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009-2010. *Environ Health Perspect.* 2016;124(1):81-87. doi:10.1289/ehp.1307909
48. Jeddy Z, Tobias JH, Taylor EV, Northstone K, Flanders WD, Hartman TJ. Prenatal concentrations of perfluoroalkyl substances and bone health in British girls at age 17. *Arch Osteoporos.* 2018;13(1):84. doi:10.1007/s11657-018-0498-5
49. Koskela A, Finnilä MA, Korkalainen M, et al. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. *Toxicol Appl Pharmacol.* 2016;301:14-21. doi:10.1016/j.taap.2016.04.002
50. Ballesteros V, Costa O, Iñiguez C, Fletcher T, Ballester F, Lopez-Espinosa MJ. Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: A systematic review of epidemiologic studies. *Environ Int.* 2017;99:15-28. doi:10.1016/j.envint.2016.10.015
51. Vucic S, Korevaar TIM, Dharmo B, et al. Thyroid Function during Early Life and Dental Development. *J Dent Res.* 2017;96(9):1020-1026. doi:10.1177/0022034517708551
52. Talia C, Connolly L, Fowler PA. The insulin-like growth factor system: A target for endocrine disruptors? *Environ Int.* 2021;147:106311. doi:10.1016/j.envint.2020.106311
53. Werner H, Katz J. The emerging role of the insulin-like growth factors in oral biology. *J Dent Res.* 2004;83(11):832-836. doi:10.1177/154405910408301102
54. López-Doval S, Salgado R, Lafuente A. The expression of several reproductive hormone receptors can be modified by perfluorooctane sulfonate (PFOS) in adult male rats. *Chemosphere.* 2016;155:488-497. doi:10.1016/j.chemosphere.2016.04.081
55. Di Nisio A, Sabovic I, Valente U, et al. Endocrine Disruption of Androgenic Activity by Perfluoroalkyl Substances: Clinical and Experimental Evidence. *J Clin Endocrinol Metab.* 2019;104(4):1259-1271. doi:10.1210/jc.2018-01855
56. Jeremias F, Pierri RAG, Souza JF, et al. Family-Based Genetic Association for Molar-Incisor Hypomineralization. *Caries Res.* 2016;50(3):310-318. doi:10.1159/000445726

- Dental enamel defects affect one in ten people worldwide
- Molar-incisor hypomineralization is a distinct condition with unclear etiology
- This is the first study to explore the contribution of background levels of POPs
- Prenatal exposure to  $\beta$ -HCH is associated with a reduced risk of enamel defects
- PCBs and PFASs are associated with increased risk in sex-stratified analysis

Journal Pre-proof

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof