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Marchela Pandelova, Raffaella Piccinelli, Walkiria Levy Lopez, Bernhard Henkelmann, Jose Manuel Molina-Molina, Juan Pedro Arrebola, Nicolas Olea, Catherine Leclercq, Karl-Werner Schramm

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**Assessment of PCDD/F, PCB, OCP and BPA dietary exposure of non-breast-fed European infants**

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Methods/Techniques:	Bioassay, Chromatography - GC/MS, Chromatography - LC/MS, Exposure assessment
Additives/Contaminants:	Bis-phenol A, Dioxins, PCBs, Pesticides - organochlorine
Food Types:	Baby food, Beverages, Infant formulae
Abstract:	The aim of the study was to obtain data on the exposure of non-breast-fed infants to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), and Bisphenol A (BPA) and its chlorinated derivatives through consumption of commercial infant

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	foods with largest shares of the market in 22 EU countries. The E-Screen bioassay was employed to assess the estrogenicity of the baby foods and the ethoxyresorufin-O-deethylase (EROD) induction was measured to determine the levels of PCDD/F and PCB. Consequently, the highest total effective xenoestrogen burden (TEXB) value of 73.60 pM Eeq/g was found in the soy-based formula and the EROD-bioassay was always below the LOQ (3.5 pg/g). Overall, the estimated dietary exposure to BPA via commercial baby foods was lower than the Tolerable Daily Intake (TDI) of 50 µg/kg body weight (bw). Furthermore, our findings indicated that the dietary exposure of 0-9-month-old infants through the products investigated here does not exceed the maximum Tolerable Daily Intake (TDI) of 4 pg WHO-TEQ (toxic equivalents)/kg bw. However, exposure to more than 2 pg WHO-TEQ/kg bw/day might occur for 0-4 month of age infants consuming "starting" hypoallergenic formula. Moreover, analysis of OCP indicated that the dietary exposure of non-breast-fed infants was not harmful. Considering the importance of early development and the vulnerability of infants and children, it is essential to determine their dietary exposure to contaminants in order to decide which efforts of risk reduction should receive highest priority.
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# 1 Assessment of PCDD/F, PCB, OCP and BPA dietary exposure of non- 2 breast-fed European infants

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## 19 20 Abstract

21 The aim of the study was to obtain data on the exposure of non-breast-fed infants to polychlorinated  
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23 pesticides (OCP), and Bisphenol A (BPA) and its chlorinated derivatives through consumption of  
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25 was employed to assess the estrogenicity of the baby foods and the ethoxyresorufin-O-deethylase  
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34 hypoallergenic formula. Moreover, analysis of OCP indicated that the dietary exposure of non-breast-

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3 35 fed infants was not harmful. Considering the importance of early development and the vulnerability of  
4 36 infants and children, it is essential to determine their dietary exposure to contaminants in order to  
5 37 decide which efforts of risk reduction should receive highest priority.  
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10 39 **Keywords:** PCDD/F; PCB; OCP; BPA; food; infant  
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## 12 41 **Introduction**

13 42 Food is generally recognized as the main source of human exposure to persistent organic pollutants  
14 43 (POPs) such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F), polychlorinated  
15 44 biphenyls (PCB) and organochlorine pesticides (OCP) (Ahlborg et al., 1995; Weijs et al., 2006).  
16 45 Several health risk assessments were used by different authorities to propose safety limits for all  
17 46 human sub-population deriving from non-carcinogenic end-points and referring to different exposure  
18 47 periods. A WHO-expert consultation in May 1998 ended with the establishment of a Tolerable Daily  
19 48 Intake (TDI) of 1-4 pg WHO-TEQ (toxic equivalents)/kg body weight (bw) (WHO, 2000). In 2001,  
20 49 the Scientific Committee on Food of the European Commission established a Tolerable Weekly Intake  
21 50 (TWI) of 14 pg TEQ/kg bw (SCF, 2001) for the European population. More, recently the Joint  
22 51 FAO/WHO Expert Committee of Food Additives established a Provisional Tolerable Monthly Intake  
23 52 (PTMI) of 70 pg WHO-TEQ/kg bw/month (JECFA, 2002).  
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34 54 Children, particularly infants, can be biologically more sensitive to certain toxicant on the body weight  
35 55 basis than adults. Current understanding of the rates of maturation of metabolism and evidence from  
36 56 case studies indicated that infants up to the age of 6 months typically lack the capacity to detoxify and  
37 57 eliminate substances as readily as adults (Dourson et al., 2004). Moreover, the Scientific Committee  
38 58 on Food of the European Commission stated that the estimated dioxin dietary exposure of breast-fed  
39 59 infants is one to two orders of magnitude higher than the estimated adult dietary exposure (SCF,  
40 60 2001). Although there is considerable evidence that the concentrations of PCDD/F and OCP in breast  
41 61 milk have fallen in recent years (Krauthacker et al., 2006; Mueller et al., 2008; Hsu et al., 2007). Since  
42 62 breast-feeding has a measurable positive influence on neurological and immunological development, a  
43 63 formula diet should not be recommended as a means of lowering dioxin intake. At the same time in  
44 64 some EU countries only about 50% of mothers initiate breast-feeding (Cattaneo, 2008). According to  
45 65 statistics from 2007 few European women breastfeed their infants exclusively at six months of age  
46 66 (EC, 2004) and only 33.1% of infants in the USA are exclusively breastfed up to 3 months of age  
47 67 (CDC). The increasing number of mothers who feed their babies industrially processed formula milk  
48 68 or solid foods as vegetables and meat or fish purée, has caused the baby market to grow significantly  
49 69 and with it, the assortment of products offered. According to the DONALD study, the sale of  
50 70 commercial infant food in Germany in 1996 was approximately 30% of the total sales of dietetic foods  
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60 71 (Kersting et al., 1998).

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3 72 Thus, commercial infant food plays an important role in the infant's diet, but few studies have  
4 73 examined the PCDD/F, PCB and OCP in formula milk consumed by infant (Hsu et al., 2007; Lorán et  
5 74 al., 2007; Chovancová et al., 2005; Ramos et al., 1989; Weijs et al., 2006; Mezcua et al., 2007).  
6 75 Additionally, it has been shown that exposure to bisphenol A (BPA) occur through the consumption of  
7 76 beverages or foods that have been in contact with polycarbonate plastic containers or epoxy resins in  
8 77 food packaging (Kubwabo et al., 2009; Maragou et al., 2006; Lopez-Espinosa et al., 2007). BPA is  
9 78 readily chlorinated by reaction with residual chlorine molecules, e.g. residual chlorine used as a  
10 79 disinfectant in drinking water (Zafra et al., 2003) or sodium hypochlorite, which is usually applied as a  
11 80 bleaching agent in paper factories (Fukazawa et al., 2002). Therefore, chlorinated BPA derivatives are  
12 81 potential contributors to inadvertent human exposure to bisphenols. In fact, tetrachlorobisphenol-A  
13 82 and lower chlorinated analogues (mono-, di- and tri-chloroBPA) were recently detected in the  
14 83 environment (Liu et al. 2009) and human tissues (Fernandez et al., 2007). In addition, chlorinated BPA  
15 84 derivatives have been shown to be estrogenic (Rivas et al., 2001), with affinities for estrogen receptors  
16 85 that are sometimes higher than those of the parent BPA compound (Takemura et al. 2005; Kitamura  
17 86 2005). In its risk assessment on BPA adopted in 2006, the European Food Safety Authority (EFSA) set  
18 87 a Tolerable Daily Intake (TDI) of 50 µg/kg bw (EFSA, 2006) for this substance. EFSA re-confirmed  
19 88 the TDI in its 2010 opinion, while pointing out some uncertainties (EFSA, 2010). Successively, the  
20 89 European Commission has announced a ban on the use of BPA in plastic baby bottles from June 2011.  
21 90 The current study, as a part of the EU funded research project CASCADE (Chemicals as contaminants  
22 91 in the food chain; Network of Excellence, FP6 funded consortium, targeting health risks in food) is  
23 92 aimed at evaluating the current dietary exposure levels of non-breast-fed European infants. By the use  
24 93 of chemical target analysis, the scientific focus has in particular been on PCDD/F, PCB, OCP and  
25 94 BPA and some of its chlorinated derivatives. For this purpose, milk-, soy- and hypoallergenic-based  
26 95 infant formulae, as well as solid foods and beverages from the EU market were examined.  
27 96 Additionally, the E-Screen bioassay was used to assess the estrogenicity of the baby foods and the  
28 97 ethoxyresorufin-O-deethylase (EROD) induction was measured for determination of PCDD/F and  
29 98 PCB. Furthermore, the dietary exposure of non-breast-fed infants of age 0-9 months of age were  
30 99 estimated and compared with the above mentioned Tolerable Daily Intakes.  
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## 50 101 **Materials and Methods**

### 51 102 *Baby food samples included in the study*

52 103 Market baskets of baby foods designed for consumption during the first 9 months of life by an  
53 104 "average" EU baby fed with infant formulae and weaned (at the 5<sup>th</sup> month) with solid foods and  
54 105 beverages were put together (Table 1). The subsequent investigations concerned an infant whose diet  
55 106 consisted entirely of commercial baby foods, i.e. who was not breast fed at all. The 2007 market data  
56 107 made available by "Food for Thought" ([www.fft.com](http://www.fft.com)) was used to identify the major brands of baby  
57 108 foods and their shares of the market in 22 EU countries considered to represent the entire EU.

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3 109 A total of 6 pooled samples of “starting” infant formulae of milk-based (Mf), soy-based (Sf),  
4 110 hypoallergenic-based (HAf) and “follow-on” infant formulae of milk-based (fMf), soy-based (fSf) and  
5 111 hypoallergenic-based (fHAf) and 5 pooled samples of solid foods and beverages (SFB) designed for  
6 112 5-9-month-old infants (SFB5, SFB6, SFB7, SFB8, and SFB9) were prepared separately for chemical  
7 113 target analysis. The proportion of each product in the pooled sample was the same as its share of the  
8 114 European market. Further details have been reported elsewhere (Piccinelli et al., 2010). In order to  
9 115 assess the potential exposure to nuclear receptor through the whole diet, another 6 pooled samples of  
10 116 fMf+SFB5, fMf+SFB9, fSf+SFB5, fSf+SFB9, fHAf+SFB5, and fHAf+SFB9 were further prepared.  
11 117 (Table 1 here)  
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19 119 Lyophilisation was performed for baby jar and beverage products using freeze drying equipment  
20 120 (Type BETA 1-16, Christ GmbH) with duration of about 36 h per sample. Consequently the fresh  
21 121 weight (fw) of 20 g solid foods and beverages for SFB5, SFB6, SFB7, SFB8, and SFB9 resulted dry  
22 122 weight (dw) of 9.44, 8.55, 8.01, 8.59, 7.87 g, respectively. The homogenisation of the pooled infant  
23 123 formulae and solid food and beverages samples was done after the lyophilisation of the separate items.  
24 124 The weighing procedures were performed in a purified glove box under nitrogen atmosphere and the  
25 125 pooled samples were further homogenised in a roto shaker (Type Reax 20/8, Heidolph GmbH) for 8 h.  
26 126 The samples were kept at room temperature and stored in brown glass jars protected from direct light.  
27 127 In order to prevent any contamination the opening and closing of the sample collector was performed  
28 128 under nitrogen atmosphere.  
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### 37 130 *Determination of PCDD/F and dioxin-like PCB amounts in baby food samples*

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39 131 Extraction of 20 g of fw infant formulae and 15 g of freeze dried solid foods and beverages was  
40 132 carried out on Accelerated Solvent Extractor (ASE 200, Dionex GmbH, Idstein, Germany) using a  
41 133 mixture of n-hexane: acetone (75:25, v/v) at 120 °C and at a pressure of 12 MPa (Cok et al., 2009).  
42 134 Two static cycles of 10 min were applied for a complete extraction. Cleanup encompassed sandwich,  
43 135 carbon and alumina chromatographic columns. PCB and PCDD/F analyses were performed by  
44 136 HRGC/HRMS. Detail information on clean-up procedure and instrumental parameters are given  
45 137 elsewhere (Cok et al., 2009). The tetra to octa PCDD/F and tetra to hepta PCB were identified and  
46 138 reported in pg WHO-TEQ/g fw. The older WHO 1998 Toxic Equivalency Factors (TEFs) values were  
47 139 used because all regulatory limits were still based on them. The lower and upper bounds approach was  
48 140 applied for quantification of the results as described elsewhere (WHO, 2003). For lower bound (LB)  
49 141 values, the concentration was set equal to zero if it was below the limit of quantification (LOQ). For  
50 142 upper bound (UB) values, the concentration was set equal to the LOQ if it was below the LOQ. So the  
51 143 UB values present the highest possible amount of a contaminant based on the analytical results. The  
52 144 analytical laboratory involved is quality assured according to DIN EN ISO/IEC 17025 and accredited  
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60 145 for the analysis of PCDD/F and PCB.

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5 147 *Determination of organochlorine pesticides (OCP) in baby food samples*  
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7 148 Extraction of 2 to 10 g of fw infant formulae and 15 g of freeze dried solid foods and beverages was  
8  
9 149 performed in the same way as for the PCDD/F. Cleanup and instrumental analysis of OCP were  
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11 150 executed according to a former described procedure (Wang et al., 2009). Only 6 of 26 OCP studied  
12  
13 151 were below the detection limit and the concentration level was presented in pg/g fw.

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16 153 *Micro-EROD bioassay*  
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18 154 Samples of 20 g of fw infant formulae and 20 g of freeze dried solid foods and beverages were Soxhlet  
19  
20 155 extracted with 800 ml toluene for 24 h. The extract was concentrated to 4 ml and then a clean-up step  
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22 156 was carried out on a sandwich column. This column consisted of 10 g active silica gel, 20 g silica gel  
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24 157 (44 % H<sub>2</sub>SO<sub>4</sub>), 40 g silica gel (4 % H<sub>2</sub>O), and 10 g Na<sub>2</sub>SO<sub>4</sub> from bottom to top. The sample was eluted  
25  
26 158 with 900 ml n-hexane. The eluate was concentrated and transferred to 200 µl DMSO under N<sub>2</sub> stream  
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28 159 at 45 °C. Subsequently, the concentrate was eluted to a mixture DMSO/Isopropanol 4:1 as an optimal  
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30 160 carrier solvent for the bioassay.

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32 161 EROD bioassay was performed using the rat liver cell line (HII4E) expressing cytochrome P4501A1  
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34 162 upon exposure to Aryl hydrocarbon receptor (AhR) agonists (Donato et al., 1993; Schwirzer et al.,  
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36 163 1999). A H4IIE cell suspension was seeded into 96 well plates and incubated for 24 hours. Following  
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38 164 this, the cells were exposed in parallel to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) standard  
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40 165 concentrations and samples keeping the ratio standard/medium and sample/medium at 0.005. Blanks  
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42 166 were also carried out. The EROD induction caused by sample extracts after 72 h of incubation  
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44 167 (persistent compounds) was compared to the dose-response curve obtained with standards of 2,3,7,8-  
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46 168 tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). The results were given as pg 2,3,7,8-TCDD toxicity  
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48 169 equivalent values (TE values) per gram sample.

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51 171 *E-Screen bioassay: TEXB assessment*  
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53 172 MCF-7 cells were used in the test of estrogenicity according to a technique slightly modified  
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55 173 (Villalobos et al., 1995) from that originally described elsewhere (Soto et al., 1992). Briefly, 0.5 ml of  
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57 174 each baby food extract including “starting” infant formulae (Mf, Sf and Hf) and pooled “follow-on”  
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59 175 infant formulae and solid foods and beverages (fMf+SFB5, fMf+SFB9, fSf+SFB5, fSf+SFB9,  
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176 fHaf+SFB5, and fHaf+SFB9) were evaporated to dryness under a gentle nitrogen stream. Then, the  
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178 177 extracts were resuspended in 10 ml of experimental medium (10% CD-FBS-supplemented phenol red-  
179  
180 178 free DMEM), vigorously shaken and left at rest for 30 min, then filtered through a 0.22 µm filter and  
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182 179 tested on MCF-7 cells at dilutions of 1:1 to 1:10,000. In each experiment, a dose-response curve (0.1  
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181 180 pM-1000 pM) of 17β-estradiol (E<sub>2</sub>), and a negative control (cell treated only with hormone-free  
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183 181 medium) were included. The bioassay was terminated on day 6 (late exponential phase) by removing  
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185 182 the media from the wells, fixing the cells and staining them with sulphorhodamine-B (SRB). The

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3 183 proliferative effect (PE) was calculated as the ratio between the highest cell yield obtained with 100  
4 184 pM of E<sub>2</sub> and the proliferation of hormone-free control cells. The PEs obtained with 1:1 to 1:10,000  
5 185 dilutions of individual extracts were converted to estradiol equivalents (Eeq) units by interpolation on  
6 186 the estradiol dose-response curve and expressed as Eeq per gram of sample, as an estimation of the  
7 187 total effective xenoestrogen burden (TEXB) (Rivas et al., 2001).  
8 188

#### 11 189 *Determination of BPA and its chlorinated derivatives in baby food samples*

12 190 An accurate and reproducible gas chromatographic-mass spectrometric (GC-MS) method was  
13 191 developed to quantify BPA and its chlorinated derivatives in infant formulae. Sample of 0.4 g of fw  
14 192 infant formula was extracted involving a protein precipitation step with acetonitrile and a solid-phase  
15 193 extraction (SPE) step using Isolute C<sub>18</sub> cartridges. After isolation, the solvent was evaporated with  
16 194 nitrogen stream and a silylation step was carried out using N,O-bis(trimethylsilyl) trifluoroacetamide  
17 195 (BSTFA) and trimethylchloro silane (TMCS) (99:1,v/v). The silylated compounds were identified and  
18 196 quantified by GC-MS using a ZB-5 MS column and selected-ion monitoring (SIM) mode. Bisphenol F  
19 197 was used as internal standard.  
20 198

21 199 Extraction of 0.5 g of freeze dried solid foods and beverages was carried out on ASE using *n*-hexane,  
22 200 followed by a liquid-liquid extraction with acetonitrile and consequent cleaning with *n*-hexane. The  
23 201 extracted sample was taken to dryness with a gentle stream of nitrogen and reconstituted with  
24 202 deionised water. A SPE step was carried out using isolute C<sub>18</sub> cartridges in order to clean up the  
25 203 sample. The solvent was removed and a silylation step was performed using BSTFA and TMCS.  
26 204 Finally, the silylated compounds were identified and quantified by GC-MS.  
27 205

28 206 The linearity of the calibration curves was tested according to the Analytical Methods Committee  
29 207 (AMC, 1994). The results for the intercept (a), slope (b), correlation coefficient (R<sup>2</sup>), and probability  
30 208 level of the lack-of-fit test and for the linearity, precision, accuracy, sensitivity, and selectivity of the  
31 209 overall assay have been presented elsewhere (Del Olmo et al., 2005; Zafra et al., 2003; Ballesteros et  
32 210 al., 2006). The limit of detection (LOD) and the LOQ for BPA and its chlorinated derivatives in infant  
33 211 formula samples ranged from 0.8 to 1.7 and 2.6 to 5.8 ng/g, respectively and from 1.5 to 3.3 and 4.9 to  
34 212 10.9 ng/g, respectively, for solid foods and beverages samples. The methodology was validated using  
35 213 recovery studies with spiked samples. Recoveries ranged from 85.3 to 108.4%, with mean values of  
36 214 99.0% (BPA), 101.2% (ClBPA), 92.9%, (Cl<sub>2</sub>BPA), 93.3% (Cl<sub>3</sub>BPA), and 93.5% (Cl<sub>4</sub>BPA).  
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#### 38 216 *Elaboration of the monthly diets for EU babies for the first 9 months of life*

39 217 The mean daily dietary exposure to PCDD/F, PCB, OCP, and BPA was calculated based on the  
40 218 estimated average amount of infant formulae (ml/day) and solid foods and beverages (g/day)  
41 219 consumed by an “average EU baby”. The values thus obtained were 25.2, 22.9, 21.0, and 18.3 g

220 (fw)/kg bw for the “starting” formulae consumed by infants 0-1, 1-2, 2-3, and 3-4 months of age,  
221 respectively; 12.8, 8.0, 4.6, 4.4 and 4.2 g (fw)/kg bw for the “follow-on” formulae consumed by  
222 infants 4-5, 5-6, 6-7, 7-8 and 8-9 months of age, respectively; and 28.8, 54.7, 72.6, 70.2 and 71.9 g  
223 (fw)/kg bw for the solid foods and beverages consumed by infants 4-5, 5-6, 6-7, 7-8 and 8-9 months of  
224 age, respectively. Further details have been reported elsewhere (Piccinelli et al., 2010).

225 The results of compound determination were converted into daily dietary exposure based on the daily  
226 consumption of the baby foods and bw of infant at a certain month, respectively. In order to establish a  
227 meaningful comparison the calculated values were additionally expressed in the same unit as the  
228 tolerable daily intakes established by international expert committees.

229

## 230 **Results and Discussion**

### 231 *Levels of PCDD/F and dioxin-like PCB and TE-EROD value baby foods*

232 Table 2 presents the LB and UB for the levels of PCB and PCDD/F in infant formulae and solid foods  
233 and beverages based on chemical analysis.

234 (Table 2 here)

235

236 No or low levels of PCDD/F or PCB were detected in the solid foods and beverages and negligible  
237 PCB levels were detected in the infant formulae. With regard to infant formulae a LB range of 0.01 to  
238 0.05 PCDD/F (WHO-TEQ) pg/g fw that correspond to 0.04 to 0.2 (WHO-TEQ) pg/g lipid (the  
239 package labelled lipid content of approximately 25 g per 100 g fw) was estimated. For comparison,  
240 slightly higher median value of total WHO-TEQ of 0.07 pg/g fw were suggested in infant formulae  
241 from the Swedish market (Broman et al., 2009). However, elevated PCDD/F level of 0.11 PCDD/F  
242 (WHO-TEQ) pg/g fw (0.44 PCDD/F (WHO-TEQ) pg/g lipid) was found for the “starting”  
243 hypoallergenic infant formula (HAf). It was also noted similar PCDD/F UB level was detected for  
244 both hypoallergenic infant formulae, i.e. HAf and fHAf. In fact, all these concentrations were lower  
245 than reported  $0.7 \pm 0.2$  pg and 0.98 (WHO-TEQ) pg/g lipid in formula milk from southern Taiwan (Hsu  
246 et al., 2007) and Slovak Republic (Chovancová et al., 2005) markets, respectively and far lower than  
247 the total median TEQ<sub>98</sub> of 15 pg/g lipid in mother milk from randomly recruited primiparas (N=183)  
248 who lived in Uppsala Country and delivered between 1996 and 2006 (Lignell et al., 2009). Recent  
249 EFSA report on monitoring of dioxin level in food and feed including “Infant and baby food” group  
250 suggested mean total dioxins equal to 0.42 pg TEQ WHO<sub>98</sub>/g lipid (EFSA, 2010).

251 The induction of EROD activity was used as a well-established biomarker for assessing the exposure  
252 to stressors such as mixture of contaminants able to elicit a response at the AhR level. However, no TE  
253 levels higher than the LOD (0.5 pg/g fw) and LOQ (3.5 pg/g fw) were observed. Therefore, the  
254 present study can not use the EROD data for further calculations of dietary exposure to dioxin-like  
255 compounds. However, the use of a bioanalytical tool is important since it allows to investigate

256 possible interactions between different chemicals present in the sample. These possible effects are not  
257 considered when single analytical determinations are performed.

258

#### 259 *Level of organochlorine pesticides (OCP) in baby foods*

260 Among of the different isomers of HCH, only  $\alpha$ -HCH in Sf and fHAf and only  $\beta$ -HCH in fHAf were  
261 detected in infant formulae (Table 3). The  $\alpha$ -,  $\beta$ -, and  $\gamma$ -isomers of HCH were present in all solid foods  
262 and beverages samples, with a monthly stepwise increase in the content of the  $\alpha$ -HCH level as the diet  
263 became more and more varied. Since the nervous system is among the vulnerable targets for lindane  
264 exposure and perinatal exposure to lindane has been associated with the development of Parkinson's  
265 disease later in life (Heusinkveld et al., 2010) the  $\gamma$ -HCH isomer (lindane) is the most important one  
266 within the HCHs. This compound was found predominantly in the solid foods and beverages samples,  
267 although still at levels much lower than the maximum residue limits (MRL) proposed for lindane i.e.  
268 0.010 mg/kg lipid (FAO/WHO, 2006).

269 (Table 3 here)

270

271 As also shown in Table 3, in almost all of the baby food samples analysed, DDTs were present at  
272 levels above the detection limit. In addition, in both types of baby food (infant formulae and solid  
273 foods and beverages) the levels of 4,4'-DDT, 2,4'-DDT, 4,4'-DDD, 2,4'-DDD and 2,4'-DDE were  
274 relatively similar. Out of the six DDTs analysed, 4,4'-DDE was present at highest levels (20.9 pg/g fw)  
275 in the milk-based formulae and in solid foods and beverages where the corresponding levels of SFB8  
276 and SFB9 were 45.5 and 37.0 pg/g fw, respectively. On a lipid basis, Mf value was 0.08 ng/g, which is  
277 far lower than the level of p,p'-DDE in breast milk reported Czech Republic 600 ng/g lipid (Cerna et  
278 al., 2010).

279

280 Heptachlor, cis-chlordane, trans-heptachlorepoxyde and endrin were not detected in any of the samples  
281 analysed (Table 3). However, trans-chlordane, oxy-chlordane and aldrin were present at negligible  
282 concentrations in solid foods and beverages (100, 60 and 40%, respectively), in particular food  
283 designed for 8- and 9-month-old infants. Although cis-heptachlorepoxyde was found in all of the baby  
284 foods, with the highest level of 0.023 ng/kg fw (0.092 ng/kg lipid) in fMf, its levels were well below  
285 the MRL of 6  $\mu$ g/kg lipid (FAO/WHO, 2006).

286

287 Among the OCP, endosulfan isomers were mostly dominant in investigated milk- and hypoallergenic-  
288 based infant formulae. The determined concentrations were 139, 132 and 148 pg/g of fw for Mf, HAf  
289 and fHAf, respectively which is below the MRL (0.1 mg/kg lipid) of endosulfan in milk (FAO/WHO,  
290 2006). Although industrialized nations have restricted or banned many organochlorine pesticides (e.g.,  
291 endosulfans), according to the available information, Spain is the main consumer of endosulfans  
292 within the European Union, accounting for almost half of the total consumption (Cerrillo et al., 2005).

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2  
3 293 In fact, 20% of samples of milk-based infant formulas from Spain were positive for this contaminant,  
4 294 which is identified as an endocrine disruptor (Mezcua et al., 2007). Also, reports on human exposure  
5 295 to persistent OCP have indicated considerable exposure to endosulfans, as the fact that they have been  
6 296 detected in placenta, umbilical cord blood and breast milk of women as well as in adipose tissue of  
7 297 children living in Southern Spain (Lopez-Espinosa et al., 2008).

8 298  
9  
10 299 The result of the study revealed that out of total 26 OCP investigated only 6 were found below the  
11 300 LOD. Although in all baby food samples investigated the detected OCP levels were much lower than  
12 301 the MRL, nevertheless, there is need of continuous monitor from infant food safety point of view and  
13 302 also time tendency after restriction and ban of some OCP. It indicates also the high degree of  
14 303 dispersion of these substances in the environment as well as the distribution of OCP in the soil-plant  
15 304 system.

16 305  
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18 306 *Estrogenicity of baby food extracts in the E-Screen bioassay*

19 307 In order to estimate the estrogenicity of the samples, a total of nine baby food extracts of “starting”  
20 308 infant formula (Mf, Sf, HAF) and pooled “follow-on” infant formulae and solid foods and beverages  
21 309 (fMf+SFB5, fMf+SFB9, fSf+SFB5, fSf+SFB9, fHAF+SFB5, and fHAF+SFB9) were tested using a  
22 310 MCF-7 cell based proliferation assay (E-Screen bioassay). The biomarker of estrogenicity (TEXB)  
23 311 was developed to measure the cumulative estrogenicity of chemicals using the E-Screen bioassay.  
24 312 Table 4 lists the TEXB values (mean and standard deviation) expressed as Eeq/g sample. Because all  
25 313 tested samples were toxic for MCF-7 cells at 1:1 dilution, due to solvent toxicity, we tested serial  
26 314 dilutions up to 1:10,000 in order to obtain measurable estrogenicity of the fractions. TEXB levels  
27 315 ranged from undetectable (<1 pM Eeq) to 73.60 pM Eeq/g, the maximal estrogenicity observed.  
28 316 (Table 4 here)

29 317  
30  
31 318 Estrogenicity was <1 pM Eeq/g in Mf and HAF samples, whereas the highest TEXB values  
32 319 corresponded to the soy-based formula samples. These findings are in agreement with recent findings  
33 320 by Riu et al. (2008) on the estrogenicity of the same soy-based formula samples, using a reporter gene  
34 321 assay based on MELN and HELN bioluminescent cells. They observed a preference for transactivation  
35 322 of ER $\beta$  versus ER $\alpha$ , a type of transactivation signal profile previously described for estrogenic  
36 323 compounds such as phytoestrogens (Escande et al., 2006). Interestingly, they confirmed the presence  
37 324 of several phytoestrogens identified as aglycones (daidzein, glycitein, and genistein) using ER $\alpha$ -based  
38 325 affinity columns. Therefore, the estrogenicity of soy-based formula samples can be assumed to derive  
39 326 mostly from natural chemicals (e.g. phytoestrogens) rather than from man-made xenoestrogens.

40 327 Concerns have been raised by the scientific community about the potential risks to infant health of  
41 328 soy-based formula consumption, due to its phytoestrogen content. Tuohy (2003), in a review of the  
42 329 conflicting evidence on the risks and benefits of soy phytoestrogens, denounced the absence of

330 adequate scientific research that quantifies the level of risk for infants. He suggested the need for a  
331 precautionary approach to be taken in relation to the potential developmental effects of the  
332 consumption of pharmacologically active compounds in infancy and childhood.

333

334 *Levels of BPA and its chlorinated derivatives in baby foods*

335 Eleven baby foods including infant formulae (Mf, Sf, HAf, fMf, fSf and fHAf,) and solid foods and  
336 beverages (SFB5, SFB6, SFB7, SFB8, and SFB9) were analysed for BPA and its derivatives and the  
337 results were presented in ng/g of fw. No detectable levels were found in infant formulae samples, with  
338 the exception of the BPA concentration (3.60 ng/g of sample) being detected in HAf. This result is in  
339 agreement with previous studies that report BPA concentration in canned infant formula samples  
340 (powdered and whole milk) ranging from <1.7 to 15.2 ng/g (Maragou et al., 2006). In addition, the  
341 concentrations reported in the present work fall within the range of previously published  
342 measurements of BPA in canned liquid infant formula from the United States (Ackerman et al., 2010)  
343 as well as Canada (Cao et al., 2008) and are much lower than the BPA levels reported in powdered  
344 infant formula purchased from the local Taiwan market that ranged from 45 to 113 ng/g (Kuo and  
345 Ding, 2004).

346

347 Regarding solid foods and beverages samples investigated the BPA levels were below the determined  
348 LOQ level of 1.5 ng/g. However, a concentration of 17.35 ng/g of sample was detected in the food  
349 designed for 9 month-old infants (SFB9). To date, several studies have been dedicated to determine  
350 BPA levels in food and via food packaging (Muncke, 2009). In general, concentrations of BPA in  
351 canned food products differ considerably among food types. For example, BPA values range from 7.8  
352 to 24.4 ng/g in canned fruits and from 55 to 103 ng/g in canned vegetables from the Spanish market  
353 (Garcia-Prieto et al., 2008). By contrast, the mean of BPA concentrations in canned vegetables from  
354 Canadian (Cao et al., 2010) and Italian markets (Grumetto et al., 2008) were relatively low (less than  
355 10 ng/g).

356

357 BPA chlorinated derivatives (ClBPA, Cl<sub>2</sub>BPA, Cl<sub>3</sub>BPA and Cl<sub>4</sub>BPA) were not detectable in SFB5,  
358 SFB6, SFB7, while these compounds were below LOQ (4.9 ng/g) in SFB8 sample, and subsequently  
359 Cl<sub>3</sub>BPA and Cl<sub>4</sub>BPA were quantified at concentrations 12.45 and 5.11 ng/g of sample, respectively, in  
360 SFB9. In general, a tendency to higher concentrations of BPA and its derivatives was found in  
361 industrial solid foods and beverages with increasing months.

362

363 *Daily intake of PCDD/F, dioxin-like PCB, OCP and BPA of non-breasted infants (0-9 month aged)*  
364 *fed with baby foods*

365 The dietary exposure to PCDD/F and PCB (pg WHO-TEQ/kg bw/day) of 0-9 month-old infants was  
366 estimated considering the LB levels in “starting” Mf, Sf, HAf (for infants 0-4 months), “follow-on”

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3 367 fMf, fSf, fHaf infant formula, respectively, and solid foods and beverages (SFB5-SFB9) and their  
4  
5 368 foods consumption (Table 5). The study does not consider the additional drinking water exposure as  
6  
7 369 well as the specific exposure at month 6<sup>th</sup> and 9<sup>th</sup> that may arise during crawling and soil ingestion.  
8  
9 370 (Table 5 here)

10 371  
11 372 PCDD/F and PCB exposure appeared to decrease from month to month, due to the reduced  
12  
13 373 consumption of formula per kg bw. Moreover, exposure to dioxin-like PCB via the six typologies of  
14  
15 374 infant formulae during the first 9 months of life was far lower than the exposure to PCDD/F (Table 5).  
16  
17 375 Exposure of 5-9 month-old infants to PCB via solid foods and beverages investigated was estimated to  
18  
19 376 be between 0 and 0.73 pg WHO-TEQ/kg bw/day, which is higher than the corresponding value for  
20  
21 377 PCDD/F from fifth to ninth month of postnatal life.

22 378  
23 379 The highest estimated exposure to PCDD/F of more than 2 pg WHO-TEQ/kg bw/day was exhibited by  
24  
25 380 infants consuming HAF who had an average body weight of less than 6.4 kg (unique LB and UB  
26  
27 381 PCDD/F concentration levels). In general, HAF is occasionally recommended by pediatricians for  
28  
29 382 babies who have a severe problem digesting cow's milk based formula or soy formula. HAF require  
30  
31 383 more intensive processing than Mf and Sf and more processing tend to introduce more contamination.  
32  
33 384 Hypoallergenic formulae are processed by enzymatic hydrolysis of different protein sources such as  
34  
35 385 bovine casein/whey and soy followed by further processing such as heat treatment and/or  
36  
37 386 ultrafiltration, or they are based on amino acid mixtures (Høst and Halcken, 2004). Exposure to dioxins  
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39 387 of infants up to two months of age through HAF is of potential concern. Furthermore, consideration of  
40  
41 388 UB PCDD/F and PCB values in Mf and Sf may suggest critical dietary exposure for same bw group  
42  
43 389 infants such as  $2.42 \pm 0.32$  and  $1.99 \pm 0.26$  pg WHO-TEQ/kg bw/day, respectively. Although the similar  
44  
45 390 UB levels for PCDD/F in HAF and fHAF, lower than 2 pg WHO-TEQ/kg bw/day exposure was  
46  
47 391 estimated during the weaning period due to differences in the pattern of consumption.

48 392  
49 393 In general, excluding hypoallergenic formula the baby food items examined may expose 0-4- and 4-9-  
50  
51 394 month-old non-breast-fed infants to approximately 1 and 0.7 pg WHO-TEQ/kg bw/day, respectively.  
52  
53 395 Similarly, the estimated chronic exposure to PCDD/F and dioxin-like PCB was 1.1 pg WHO-TEQ/kg  
54  
55 396 bw/day for infants 5 months of age who consume infant formulae and vegetables available on the  
56  
57 397 Dutch market (Weijs et al., 2006). Overall, the dietary exposures estimated here do not exceed the  
58  
59 398 maximum TDI of 4 pg WHO-TEQ/kg bw/day and moreover, are orders of magnitude lower than  
60  
401 399 reported total TEQ daily intake for breast-fed infants from Czech Republic 117- and 271 pg/kg bw/day  
402 400 (Bencko et al., 2004), Turkey 37- and 70 pg/kg bw/day (Cok et al., 2009) and Spain 49.6 pg/kg  
401 bw/day (Bordajandi et al., 2008).

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2  
3 403 BPA level was detected only in the HAF out of the six infant formulae samples analysed.  
4  
5 404 Consequently, the estimated dietary exposure to BPA of 0.09, 0.08, 0.08 and 0.07  $\mu\text{g}/\text{kg}$  bw/day for 0-  
6  
7 405 1, 1-2, 2-3 and 3-4 month aged infants are referred only to infants fed with this particularly infant  
8  
9 406 formulae. These dietary exposures are lower than the established TDI (50  $\mu\text{g}/\text{kg}$  bw/day) for BPA.  
10  
11 407 Regarding dietary exposure during the weaning period, a value of 1.7  $\mu\text{g}/\text{kg}$  bw/day for 8-9 months of  
12  
13 408 age infants was estimated. For solid foods and beverages consumed in the fifth and eighth months of  
14  
15 409 life, the BPA and chlorinated derivatives levels were lower than the determined LOQ range.

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17 410  
18 411 In general, only low levels of OCP were detected in the samples of baby food investigated. The  
19  
20 412 highest DDT daily exposure 0.0001  $\mu\text{g}/\text{kg}$  bw was estimated for 4-5- month-old infants fed fMf, a  
21  
22 413 value below the limit of 10  $\mu\text{g}/\text{kg}$  bw/day (FAO/WHO, 2000). Indeed, the daily dietary exposure to  
23  
24 414 DDT varies considerably among regions. The lowest dietary exposure has been observed in countries  
25  
26 415 where restriction and ban of DDT had been executed earliest (Sweden, Germany and Norway). Due to  
27  
28 416 later termination of production and restricted use of DDT, higher dietary exposure was reported in  
29  
30 417 Poland, the Czech Republic and Russia. In this regard, our results seems to be comparable with the  
31  
32 418 Canadian data (Hoover, 1999) for infants consuming breast milk, but lower than the dietary exposures  
33  
34 419 of 8  $\mu\text{g}/\text{kg}$  bw recently estimated in Poland (Szyrwińska and Lulek, 2007) or Czech Republic (Cerna  
35  
36 420 et al., 2010) daily.

37  
38 421  
39 422 With regard to  $\alpha$ -,  $\beta$ - and  $\gamma$ - HCH, the worst-case scenario would involve exposure to 0.6, 0.02 and 0.6  
40  
41 423 ng/ kg bw/day for infants consuming solid foods and beverages during the eighth month (BFB8), fHAF  
42  
43 424 during the fourth month and solid foods and beverages during the fifth month of life (BFB5),  
44  
45 425 respectively. However, all of these values are also very low compared to the Tolerable Daily Intakes  
46  
47 426 (TDI) of 5, 1 and 12.5  $\mu\text{g}/\text{kg}$  bw/day, respectively (DFG, 1982).

## 427 428 **Conclusions**

429  
430 429 The present assessment of levels of four classes of endocrine disrupting compounds: PCDD/F, PCB,  
431  
432 430 OCP and BPA and its derivatives in formulae and solid foods and beverages designed for infants  
433  
434 431 focused on the products with the largest shares of the market in 22 EU countries. In addition, the  
435  
436 432 dietary exposure to these contaminants in the first 9 months of life of non-breast-fed infants was  
437  
438 433 estimated. The results were compared with established tolerable daily intakes. The established special  
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439 434 regulations for infants and children nutrition were also considered whereas here presented scientific  
440  
441 435 results could be used to improve our current knowledge on potential exposure to nuclear receptor  
442  
443 436 modulators through calculation in the different scenarios. Although direct analyses in human  
444  
445 437 biological samples (e.g. urine, blood) are known to be the best estimators of total exposure to some of  
446  
447 438 these contaminants, the aim of the present study was to identify and quantify potential sources of  
448  
449 439 exposure in infant diet. In sum the following findings were found:

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3 440 a. Using chemical target analysis the levels of PCDD/F and PCB in baby foods were low, with  
4 highest value of 0.11 pg/g (0.44 pg/g lipid) being detected in HAF. The sensitivity of the  
5 441 EROD bioassay was not high enough to calculate possible AhR inducer intakes at these low  
6 442 levels.  
7 443  
8  
9 444 b. Due to infant high food consumption per kg bw of the infants, the determined low PCDD/F  
10 445 and PCB concentrations in baby foods may contribute to a dietary exposure of 0.14 to 1.27 pg  
11 446 WHO-TEQ/kg bw/day for 0-9 months non-breastfed infants. The study notified that 0-4  
12 447 months aged infants consuming HAF may have dietary exposure of more than 2 pg WHO-  
13 448 TEQ/kg bw.  
14  
15 449 c. Only 6 of 26 OCP studied were found below the LOD, implying a wide exposure of this  
16 450 susceptible population, even within the MRL.  
17  
18 451 d. TEXB assessment resulted in an estrogenicity in the range from undetectable (<1 pM Eeq/g)  
19 452 to 73.60 pM Eeq/g (maximal estrogenic activity), the latter for soy-based formula samples,  
20 453 therefore attributable to phytoestrogens content.  
21  
22 454 e. Among investigated baby foods BPA was only found in HAF (3.60 ng/g) and in solid foods  
23 455 and beverages designed for 9 month-old infants (17.35 ng/g). In addition, the Cl<sub>3</sub>BPA and  
24 456 Cl<sub>4</sub>BPA concentrations were only detected in solid foods and beverages designed for 9 month-  
25 457 old infants at levels of 12.45 and 5.11 ng/g of sample, respectively.  
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34 459 Finally, the levels of contaminants detected in major commercial baby food products sold in the EU  
35 460 and the estimated dietary exposure by non-breast-fed infants are lower in magnitudes than those  
36 461 reported for nursing infants. However, and more importantly, low exposure to environmental  
37 462 chemicals, especially in the first months of life, must also take account of possible additive effects of  
38 463 mixture of chemicals. Indeed combinations may cause an effect even at concentrations of each  
39 464 individual chemical that alone would not produce measurable consequences. Further research is  
40 465 warranted to investigate and interpret the levels of endocrine disruptors. These results should provide a  
41 466 basis for recommendations concerning the risks posed by commercial baby foods to the health of  
42 467 young children.  
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Table 1. Infant age for inclusion of “infant formulae” and “baby foods and beverages”

Foods	Months								
	1	2	3	4	5	6	7	8	9
“Infant formulae”									
“Starting” infant formula	X	X	X						
“Follow - on” infant formula				X	X	X	X	X	X
“Baby foods and beverages” (BFB)									
Vegetables / potatoes					X	X	X	X	X
Fruit / fruit juice					X	X	X	X	X
Cereals:									
Infant cereals without gluten					X	X	X	X	X
Infant cereals with gluten						X	X	X	X
Infant cereals with milk						X	X	X	X
Pasta							X	X	X
Biscuits								X	X
Meats					X	X	X	X	X
Pulses						X	X	X	X
Dairy based dessert with fruit							X	X	X
Fish								X	X
Dairy based dessert with chocolate									X

Table 2. Lower bound (LB) and upper bound (UB) concentration levels of PCDD/F and PCB (WHO-TEQ) pg/g fw in “infant formulae” and “baby foods and beverages” samples

Type baby food		Mf	Sf	HAF	fMf	fSf	fHAF	Baby foods and beverages				
Infant age (month)		0 - 4 <sup>th</sup> month			up to the 5 <sup>th</sup> month			5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>
PCDD/F (WHO-TEQ) pg/g fw	Lower bound (LB)	0.04	0.05	0.11	0.04	0.02	0.01	n.d.	0.0035	0.00001	0.00002	0.0005
	Upper bound (UB)	0.09	0.09	0.11	0.09	0.07	0.12	0.03	0.0436	0.0384	0.0640	0.0693
PCB (WHO-TEQ) pg/g fw	Lower bound (LB)	0.0010	0.0003	0.0005	0.0030	0.0001	0.0006	0.0000	0.01	0.01	0.01	0.01
	Upper bound (UB)	0.0021	0.0013	0.0006	0.0031	0.0013	0.0017	0.17	0.01	0.01	0.01	0.01

fw: fresh weight; n.d.: not detectable; Mf: “starting” infant formulae of milk-based; Sf: “starting” infant formulae of soy-based; HAF: “starting” infant formulae of hypoallergenic-based; fMf: “follow on” infant formulae of milk-based; fSf: “follow on” infant formulae soy-based; fHAF: “follow on” infant formulae hypoallergenic-based

Table 3. OCP concentration level (pg/g fw) in “infant formulae” and “baby foods and beverages” samples

Substance	Mf	Sf	HAF	fMf	fSf	fHAF	BFB5	BFB6	BFB7	BFB8	BFB9	LOD (mean±STD)	LOQ (mean±STD)
$\alpha$ -HCH	n.d.	2.7	n.d.	n.d.	n.d.	2.3	4.3	6.6	6.9	8.0	7.6	1.0±0.9	2.0±1.8
$\beta$ -HCH	n.d.	n.d.	n.d.	n.d.	n.d.	1.7	1.1	0.9	1.5	1.4	1.2	2.0±2.6	4.0±5.2
$\gamma$ -HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22.7	10.7	13.3	17.4	13.4	1.6±1.8	2.9±3.6
$\delta$ -HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.1±2.7	4.1±5.4
$\varepsilon$ -HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.3±0.8	2.3±1.7
Pentachlorobenzene	n.d.	4.2	7.3	n.d.	n.d.	6.4	0.28	3.7	8.2	27.4	122	0.2±0.2	0.4±0.4
Hexachlorobenzene	n.d.	69.9	60.8	n.d.	n.d.	45.9	n.d.	n.d.	n.d.	0.52	8.9	1.8±1.8	3.5±3.5
4,4'-DDT	n.d.	5.8	1.3	7.6	8.6	1.8	2.1	1.9	2.2	5.6	3	0.6±0.6	1.2±1.2
2,4'-DDT	2.9	1.6	n.d.	2.7	1	0.84	0.9	0.8	0.9	1.9	1.1	0.6±0.6	1.2±1.2
4,4'-DDD	4.2	3.2	5.2	10.8	5.3	3.1	0.7	0.7	1.0	4.6	3.9	0.3±0.3	0.5±0.5
2,4'-DDD	2.4	0.84	1.5	2.4	1.7	0.84	0.1	0.1	0.1	1.3	0.9	0.2±0.2	0.5±0.5
4,4'-DDE	20.9	1.9	10.1	16.5	4.9	11.2	8.6	10.1	9.4	45.5	37.0	0.7±0.8	1.4±1.5
2,4'-DDE	4.0	0.67	0.61	n.d.	0.72	0.84	n.d.	n.d.	n.d.	0.3	0.2	0.6±0.7	1.1±1.3
<i>trans</i> -Chlordan	n.d.	1.8	n.d.	n.d.	n.d.	n.d.	0.7	0.3	0.3	0.3	0.4	0.2±0.1	0.4±0.3
<i>cis</i> -Chlordan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2±0.1	0.4±0.3
<i>oxy</i> -Chlordan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.4	0.3	1.0±1.4	1.9±2.9
Heptachlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5±0.6	0.9±1.2
<i>cis</i> -Heptachlorepoxyd	10.9	9.7	2.2	23	11.2	1.5	1.3	1.6	1.5	1.4	1.3	0.4±0.6	0.8±1.2
<i>trans</i> -Heptachlorepoxyd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5±2.2	3.0±4.4
Aldrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.2	0.9±1.3	1.8±2.6
Dieldrin	8.5	27	3.9	18	25.2	6.4	5.7	8.1	7.0	4.7	4.2	1.0±1.0	2.0±2.0
Endrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6±2.4	5.1±4.8
Endosulfan-I	74.5	34.3	101.0	89.9	33.9	116	15.3	22.4	17.8	19.5	16	2.2±2.6	4.5±5.3
Endosulfan-II	139.0	39.6	132.0	83.9	61.8	148	35.5	37.0	27.4	33.2	24.9	2.1±2.0	4.2±3.9
Methoxychlor	n.d.	0.82	n.d.	n.d.	1.4	n.d.	0.4	0.3	0.5	0.6	0.5	0.8±0.5	1.6±1.0
Mirex	n.d.	n.d.	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2±0.2	1.5±0.3

fw: fresh weight; n.d.: not detectable; STD: standard deviation; Mf: “starting” infant formulae of milk-based; Sf: “starting” infant formulae of soy-based; HAF: “starting” infant formulae of hypoallergenic-based; fMf: “follow on” infant formulae of milk-based; fSf: “follow on” infant formulae soy-based; fHAF: “follow on” infant formulae hypoallergenic-based; BFB5–BFB6–BFB7–BFB8–BFB9: baby foods and beverages available for month 5, 6, 7, 8, and 9 respectively

Table 4. The amount of the total effective xenoestrogen burden (TEXB) of baby food extracts in E-Screen bioassay

	TEXB (pM Eeq/g of sample)
Mf	0.25 ± 0.11
HAf	0.44 ± 0.22
Sf	70.13 ± 9.81
fMf+ BFB5	0.22 ± 0.15
fHAf+ BFB5	0.31 ± 0.18
fSf+ BFB5	73.60 ± 6.22
fMf+ BFB9	0.29 ± 0.15
fHAf+ BFB9	0.85 ± 0.31
fSf+ BFB9	69.25 ± 8.21

Mf: “starting” infant formulae of milk-based; Sf: “starting” infant formulae of soy-based; HAf: “starting” infant formulae of hypoallergenic-based; fMf: “follow on” infant formulae of milk-based; fSf: “follow on” infant formulae soy-based; fHAf: “follow on” infant formulae hypoallergenic-based; BFB5 and BFB9: baby foods and beverages available for month 5 and 9, respectively

Table 5: Estimated dietary exposure to PCDD/F and PCB (pg WHO-TEQ/ kg bw/day) for non-breasted infants 0-9 months of age fed with baby foods available on the EU market

Type baby foods	Infant age (month)								
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
PCDD/F TEQ (pg/kg bw)/day									
Milk infant formula	1.00	0.92	0.84	0.73	0.51	0.32	0.18	0.18	0.17
Soy infant formula	1.26	1.15	1.05	0.91	0.26	0.16	0.09	0.09	0.09
Hypoallergenic infant formula	2.78	2.52	2.31	2.01	0.13	0.08	0.05	0.04	0.04
Baby foods and beverages					0.00	0.19	0.00	0.00	0.04
PCB TEQ (pg/kg bw)/day									
Milk infant formula	0.03	0.02	0.02	0.02	0.04	0.02	0.01	0.01	0.01
Soy infant formula	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00
Hypoallergenic infant formula	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
Baby foods and beverages					0.00	0.55	0.73	0.70	0.72
Total TEQ (pg/kg bw)/day									
Milk infant formula	1.03	0.94	0.86	0.75	0.55	1.08	0.92	0.89	0.94
Soy infant formula	1.27	1.16	1.06	0.92	0.26	0.90	0.82	0.79	0.85
Hypoallergenic infant formula	2.79	2.53	2.32	2.02	0.14	0.82	0.78	0.74	0.80