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# Infant dietary exposure to dioxins and dioxin-like compounds in Greece

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ABSTRACT

The dietary exposure of infants to polychlorinated dibenzo dioxins and furans (PCDD/Fs) and dioxin like polychlorinated biphenyls (dl-PCBs) is an issue of great social impact. We investigated for the first time the dietary intake of these compounds in infants living in Greece. We included in our study two age groups: 0–6 months, when infants are fed exclusively by human milk and/or formula milk, and 6 to 12 months, when solid food is introduced to nutrition. We took into consideration analytical results for PCDD/Fs and dl-PCBs concentrations in the most popular infant formulae in the Greek market, previous data for mother milk concentrations of PCDD/Fs and dl-PCBs from Greece, and finally analytical data for fat-containing food products from the Greek market. In the first study group, it was found than in infants exclusively fed by breast milk, the calculated sum of PCDD/Fs and dioxin-like PCBs (60.3–80.4 TEQ pg/kg body weight) was significantly higher than that of infants that consume a combination of human milk and formula (31.2–41.6 TEQ pg/kg body weight). In the second study group, separate daily intake estimations were performed for babies receiving human milk (estimated total daily intake 19.76–24.95 TEQ pg/kg body weight) and formula milk (estimated total daily intake 1.60–2.24 TEQ pg/kg body weight). The risks of this exposure should not be overestimated because nursing is restricted to a limited period of human life and besides, the potential consumption of higher levels of dioxin-like compounds is fully compensated by the significant benefits of breast-feeding.



## 1. Introduction

The dietary exposure of infants to environmental contaminants is a crucial issue of great social impact and is the subject of many investigations. It has been established that infants aged 6 months or younger have a decreased capacity to detoxify and eliminate toxic substances compared to adults (Dourson et al. 2004). In addition, the energy requirements of infants and children per kilogram of body weight are much higher than those of normal adults (Commission of the European Communities, 1993), which means that by consuming a contaminated product they are exposed to higher quantities of contaminants. Of particular concern is the exposure of infants and young children to toxic substances that exhibit reproductive and developmental toxicity since these have significant impact to their still developing immunological, neurological, reproduction systems etc.

An important group of such compounds are polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like i.e. non-ortho and mono-ortho polychlorinated biphenyls (dl-PCBs). This large family of compounds, commonly, though incorrectly, referred to collectively as "dioxins" have been classified as persistent organic pollutants (POPs), because they persist in the environment and bioaccumulate, adversely affecting the ecosystem and possibly human health. It has been found that chronic exposure of humans to these compounds causes serious adverse health effects including chloracne, reproductive and developmental effects, neurological and behavioral effects, immunotoxicity, and carcinogenicity (WHO, 1998; ATSDR Tox Profiles, 2002). Specifically for infants, health effects include reduced fetal and neonatal growth (Rylander et al., 2000), neonatal thyroid function disruption (Pluim et al., 1993), behavioral effects in early childhood (Walkowiak et al., 2001), immunologic effects in children (Weisglas-Kuperus et al., 2000).

It has been established that over 90% of human exposure to these compounds is due to food intake (Liem et al., 2000). Factors affecting PCDD/Fs and dl-PCBs levels in humans are eating habits, environmental exposure, age, sex, severe change of weight, and breast-feeding. Due to their lipophilicity dioxins and dioxin-like PCBs tend to accumulate in adipose tissue (Froescheis et al., 2000; Startin and Rose, 2003). A correlation between serum and adipose tissue, whole blood and adipose tissue, and whole blood and mother milk has been demonstrated (Patterson et al., 1988; Schecter et al., 1991; Paepke, 1998).

The burden of food products by dioxins is expressed in terms of World Health Organization Toxic Equivalents (WHO-TEQs). Each one of the dioxin or dioxin-like compounds (7 PCDDs, 10 PCDFs

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and 12 PCBs) has been assigned a Toxic Equivalency Factor, assuming as reference that the most toxic of these compounds, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) by definition has a TEF of one (Pohjanvirta and Tuomisto, 1994; Van den Berg et al. 1998). The concentration of each compound multiplied by its TEF is the Toxic Equivalency (TEQ), and the sum of TEQs of compounds detected in a sample expresses its overall toxicity. In 2005, WHO re-evaluated TEF values by introducing TEFs-2005 (Van den Berg et al., 2006).

In addition, several authorities have proposed safety limits for tolerable intake of PCDD/Fs and PCBs, in a daily basis (tolerable daily intake (TDI), 1–4 pg WHO-TEQ (toxic equivalents) kg/body weight (bw) (WHO, 1998), a weekly basis (tolerable weekly intake (TWI), 14 pg TEQ kg/bw (SCF, 2001), and a monthly basis (tolerable monthly intake (PTMI), 70 pg WHO-TEQ kg/bw (JECFA, 2002). It should be noted that tolerable intake is calculated for life-long exposure and not for a limited period of time such as infancy.

The European Union has set maximum limits for PCDD/Fs and the sum of PCDD/Fs and dl-PCBs in food products, and the set limits have been specified in EC Regulations since 2001. A number of amendments have been made to the original regulation, the most recent being EC Regulation 1259/2011. In this Regulation, maximum levels for foods for infants and young children are set as well.

Although these safety limits are applicable to adults, it is questionable whether they can be applied to children and especially to infants, which have a different metabolism leading to different tolerance to toxic substances. Another important factor differentiating the dietary exposure of infants to PCDD/Fs and dl-PCBs from that of adults is breast-feeding, which is widely promoted by the WHO (Horta et al., 2007) and recommended by pediatricians in all western countries because of its benefits for the physical and mental development of infants. However, studies in several countries have shown that breast milk contains non-neglible levels of PCDD/Fs and dl-PCBs (Chao et al., 2004; Schuhmacher et al., 2004; Li et al., 2009; Costopoulou et al., 2006; Yang et al., 2002). A time-related decrease in the concentrations of PCDD/Fs and dl-PCBs in breast milk has been reported in several studies reviewed by Ulaszewska et al., 2011, probably as a result of the application of strict regulations for the decrease of dioxin concentrations in the environment and food. Other studies have included more comprehensive samples of food products consumed by babies, such as market baskets of infant formula and other commercial packaged food including all food categories consumed by infants (Bergkvist et al., 2008; Saito et al., 2008; Piccinelli et al., 2010; Pandelova et al., 2011; Weijs et al., 2006).

In this study we undertook the task of investigating for the first time the dietary intake of PCDD/Fs and dl-PCBs in infants living in Greece. We took in consideration the specific dietary habits of Greek families and the dietary guidelines given to parents by pediatricians in Greece. We included two age groups: 0–6 months, for which human breast milk and formula milk were examined, and 6–12 months, where solid food is also introduced to infant nutrition. A key point that differentiates Greece from similar countries of the EU that have already been studied, is the fact that fewer commercial baby food is consumed, and preference is usually given to baby food preparations based on fresh meat, vegetables, fruit and dairy products, conforming in the general with the Mediterranean diet and culture. For this reason, we included in our study previous data for mother milk concentrations of PCDD/Fs and dl-PCBs from Greece, analytical results for PCDD/Fs and dl-PCBs concentrations in the most popular infant formulae in the Greek market and finally data collected by the annual programs for the official control of dioxins and dioxin-like compounds in Greek food products that have been conducted since 2002 by the Mass Spectrometry and Dioxin Analysis Laboratory, the National Reference Laboratory (NRL) for Dioxins and PCBs of Greece.

## 2. Experimental

### 2.1. Sampling

All food items were sampled by the Hellenic Food Authority within the national food safety control programs for the years 2002–2010. Sampling was scheduled to cover the whole country and was performed by the prefectures of Attiki, Central Macedonia, East Macedonia, Thessalia, Epirus, North Aegean, Peloponisos and Crete. Information regarding sampling sites and number of samples collected from each site are given in Table 1. The number of samples collected was decided based on the production characteristics (type and quantity of products) of each area. Samples analyzed were typical of infant diet in Greece and included infant formulae, baby food based on cereal, beef, poultry and lamb meat, and eggs. Each sample collected was analyzed separately, according to the procedures described in the following sections for lipid extraction, clean-up and instrumental analysis. Results (lipid content, congener concentrations and total TEQ) are presented in Table 2.

The most popular brands of formula milk were included in the study. Eight brands of formula milk for the age group 0–6 months and 10 brands for the age group 6–12 months were analyzed in this study. Samples were collected from the market and stored at room temperature (according to the producers' instructions) in the market container until analysis. Each sample was extracted and analyzed separately, after dilution of the milk powder in purified water using a magnetic stirrer. Results (lipid content, congener concentrations and total TEQ) are presented in Table 3.

Fruit and vegetables were not included, due to their low fat content that leads to minimal contribution to the dietary intake of dioxins and dioxin-like compounds. Moreover, the European Union has set limits for dioxin and dioxin-like compounds in fat containing food products (Commission Regulation 1259/2011/EC) but no EU limits have been set for fruit and vegetables. In these two groups of food, pesticides are the main factors contributing to infant exposure.

Samples were collected according to European Commission Regulation 2006/1883/EC.

Human milk samples were collected in glass containers by breast-feeding volunteers, between the third and eighth week after delivery (Costopoulou et al., 2006). All volunteers lived in the wider area of Athens, whose residents represent about half the population of Greece. All the participants provided an informed consent and were asked to complete a questionnaire concerning age, weight before pregnancy and dietary habits. Eight human milk samples were collected. TEQ values determined were 3.43–11.28 pg/g fat WHO-TEQ for PCDD/Fs (mean = 7.27, SD = 2.87) and 3.26–14.39 pg/g fat WHO-TEQ for dioxin like PCBs (mean = 5.57, SD = 3.89). Detailed information about fat content, congener concentrations and total TEQ are presented in Table 3.

### 2.2. Materials

All solvents used were residue analysis picograde and were purchased from Promochem (Germany). Carbosphere 80/100 mesh was purchased from Alltech and cleaned by elution with methanol and consequently with toluene for several weeks before use. Alumina was Basic activity Super 1 for dioxin analysis, MP Biochemicals GmbH. The sulfuric acid impregnated silica gel was prepared as follows: Silica gel (100 g, 60–200 mesh, Merck) was activated in an oven at 200 °C for at least 2 days and then mixed with concentrated sulfuric acid (44 g). The internal quantification standards used were $^{13}$C-labelled solutions of PCDD/Fs and PCBs in toluene and were added to each sample prior to clean up. They contained