PCDD/F AND DIOXIN-LIKE PCB LEVEL IN INFANT FORMULAE AVAILABLE ON THE EU MARKET

Pandelova M[1], Kasham S[1], Henkelmann B[1], Leclercq C[2], Piccinelli R[2], Schramm K-W[1,3]

[1]Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Ecological Chemistry, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany; [2]National Research Institute for Food and Nutrition, Rome, Italy; [3]TUM, Wissenschaftszentrum Weihenstephan für Ernährung und Landnutzung, Department für Biowissenschaften, Weihenstephaner Steig 23, 85350 Freising, Germany

Abstract

Human health risk assessments for dioxins and dioxin-like PCBs recommend health based exposure limits within the range of 1-4 WHO-TEQ/kg bw per day[1]. The Scientific Committee on Food of the European Commission stated that on a body weight basis, the dioxin dietary exposure of breast-fed infants has been estimated to be one to two orders of magnitude higher than the average adult dietary exposure[2]. As breast-feeding has measurable benefits for neurological and immunological development, formula feeding cannot be recommended as an alternative to lower dioxin dietary exposure. In many parts of Europe mothers are increasingly reluctant to breastfeed their babies. The main objective of the current study aims at assessing the level of protection of non-breastfed children's health during their early development. Six pooled samples of infant formulae: "starting"(aged 0–4 months) and "follow on" (after 4 months) of milk formula, soy formula and hypoallergenic formula, from the EU market basket were prepared by the EU- funded research project CASCADE Network of Excellence. HRGC/HRMS was used for analysis of PCDD/F and PCB in infant formulae. In summary, only relatively low levels of PCDD/F and PCB has been determined in infant formulae available on the EU market.

Introduction

Exposure to PCDD/F (polychlorinated dibenzo-p-dioxins and dibenzofurans) and dioxin-like PCB (dl-PCBs, i.e. polychlorinated non-*ortho* and mono-*ortho* biphenyls) occurs predominantly via animal fats in the diet[3]. Infants have a relative high dietary exposure to these substances, due to their high food consumption per kilogram body weight. Due to the fact that endocrine disruptors affect the development of the body's vital organs and hormonal systems, infants, children and developing foetuses are more vulnerable to exposure. In infant period, breast milk and formula milk are the major food sources. In general, breast fed infants have the highest dietary exposure to PCDD/F, however a considerable amount of data exits for concentrations of PCDD/Fs in human milk that reveals a decrease in levels over the last years[4]. Although the WHO recommends breastfeeding as the feeding choice for babies, infant formulae are an alternative to breast-milk that can play an important role in the infant's diet, therefore their potential contamination with PCDD/Fs is of public concern. However, most of the studies focus on the levels of PCDD/F in human milk, but only few studies investigated the PCDD/F level in formula milk for infant[5,6]. In a study carried out in the metropolitan Tokyo area, there was some evidence that the main source of dioxins was infant formulae in early stage of the weaning period whereas in the subsequent weaning stage, the main source was protein-based foods[7]. Recently in some EU countries an increasing percentage of mothers do not breastfeed their babies and only about 50% of mothers initiate breastfeeding[8]. Since the first year of life is a very vulnerable and sensitive period in the human development, the composition of infant formulae and its consumption pattern are crucial. Therefore, the EU- funded research project CASCADE (Chemicals as contaminants in the food chain; Network of Excellence, FP6 funded consortium, targeting health risks in food) decided to put an effort in revealing how European infants are fed.

The aim of the present study is to assess the dietary exposure to PCDD/F and dioxin like PCB of infant exclusively fed with infant formulae available on the EU market.

Materials and Methods

Infant formulae products included in the study

The case under study is that of an infant diet completely based on commercial infant formulae where the infant would not be breast fed at all. In order to sample the products, EU market baskets were created. To design the

baskets, the diets were elaborated monthly and 6 typologies of infant formulae were considered ("starting" and "follow on" of milk formula, soy formula and hypoallergenic formula, respectively).
In order to identify the products to be included in the market baskets, market share data from 2007 of "Food for Thought" (www.fft.com) were used[9]. The name of the holding companies and of their main brands in 22 EU countries was obtained together with their overall market share. The 22 countries included were taken to represent the whole EU. The first 8 holding companies that altogether constitute over 80% of the EU market of infant formulae were identified. The websites of these brands were searched or the companies were contacted directly in order to find the names of the products to be sampled. Finally 6 market baskets were designed and 62 different infant formulae were sampled from 8 different countries including France, Germany, Italy, Portugal, Slovakia, Spain, Sweden, UK.
The mean daily dietary exposure to dioxin-like PCB (WHO-TEQ/kg bw per day)[1] through infant formula was calculated based on the estimated average amount of "Infant formulae" (ml/day) consumed by infants.
For infants in the first 4 months of life the estimated energy requirements (kcal/kg of body weight/day) set by FAO[10] were multiplied by the standard values of weight for age and sex established by WHO[11] for formula-fed infants. The estimated energy was translated in volume of infant formula by considering energy content of 63kcal/100 ml (average energy content of infant formula available on the market, according to the labels). As a result, it was estimated that the consumption of infant formula is respectively 724 ml/day, 840 ml/day, 910 ml/day, 883 ml/day during the first, second, third and fourth month of age.
For the 5^{th}, 6^{th} and 7^{th} month of age, average consumption of infant formula was taken from the recommendations of the report of Scientific Committee on Food[12]: respectively 600 ml/day, 400 ml/day and 240 ml /day.
Ultimately the identified amounts of liquid infant formulae (ml/day) was translated in gram/day, according to the label of the sampled products, that suggests to use on average 30 ml of water to dilute 4.6 g of powder of infant formula and based on an estimated volumetric density of infant formula of 1.03 g/ml[13]. The estimated consumption of powder is therefore 99 g/day, 115 g/day, 125 g/day, 121 g/day, 82 g/day, 55 g/day, 33 g/day, 33 g/day, and 33 g/day respectively in the first 9 months of the life. These values correspond to respectively 32.2, 28.1, 24.6, 20.0, 11.7, 7.8, 4.6, 4.1 and 4.1g/kg body weight.

Pooling and homogenisation of the infant formulae samples
A total of six pooled samples of "starting" infant formulae of milk-based (Mf), soy-based (Sf) and hypoallergenic–based (HAf) and "follow on" infant formulae of milk-based (fMf), soy-based (fSf) and hypoallergenic–based (fHAf) were prepared, respectively. The weighting procedures were performed in a purified glovebox under nitrogen atmosphere and the pooled infant formulas were further homogenised in a rotoshaker, Type Reax 20/8 (Heidolph GmbH) for 8 h. The samples were kept at room temperature and stored in brown glass jars protected from direct light. In order to prevent any contamination, each further opening or closure of the sample collector was performed under nitrogen atmosphere.

Determination of PCDD/F and dioxin-like PCB amounts in infant formulae samples
Extraction of 20 g of infant formula sample was carried out using an Accelerated Solvent Extractor (ASE 200, Dionex GmbH, Idstein, Germany)[14]. The procedure was performed by using a mixture of n-hexane:acetone (75:25, v/v) at 120 °C and at a pressure of 12 MPa. Two static cycles of 10 min were applied for a complete extraction. Cleanup encompassed sandwich, alumina and florisil chromatographic columns. PCB and PCDD/F analysis were performed with a high-resolution mass spectrometer Finnigan MAT 95S (Thermo Electron GmbH, Bremen, Germany) coupled with an Agilent GC 6890 (Agilent Technogies, Palo Alto, CA, USA). The tetra to octa PCDD and PCDF and tetra to hepta PCB were identified and quantified in pg WHO-TEQ/ g infant formula sample. The enforcing lab is operating a quality assurance system according to DIN EN ISO/IEC 17025 and is accredited for the analysis of PCBs and PCDD/Fs.

Results and Discussion
The results in Table 1 present the concentration level of the PCB and PCDD/F (WHO-TEQ) pg/g infant formula sample. In general it was found that all samples contained relatively low amounts of the investigated organic toxic pollutants.

Table 1
Concentrations of PCDD/F and PCB (WHO-TEQ) pg/g of infant formula samples

sample	PCB (WHO-TEQ) pg/g sample	PCDD/F (WHO-TEQ) pg/g sample
Mf	0.001	0.04
Sf	0.0003	0.05
HAf	0.0005	0.11
fMf	0.003	0.04
fSf	0.0001	0.02
fHAf	0.0006	0.01

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

The calculated mean dietary exposure to dioxin-like PCB from the six typologies of infant formula in the first 9 months of life is low (Table 2). However based on concentration of PCDD/F estimated in the present study and on the estimated consumption of infant formula only, the dietary exposure to PCDD/F for 1 to 9 months old infant is higher than TDI minimum safety value of 1 WHO-TEQ/kg bw per day. This value was exceeded when considering all six typologies of infant formulae. Estimated dietary exposure was particularly high in the first 4 months of life for infants consuming HAf, especially in the first month of life (Table 3).

Table 2
Calculated mean dietary exposure to dioxin-like PCB from the 6 typologies of infant formulae in the first 9 months of life (pg/kg bw per day)

Months	PCB					
	Mf	Sf	HAf	fMf	fSf	fHAf
	WHO-TEQ/kg bw per day					
1	0.0242	0.0073	0.0121			
2	0.0227	0.0068	0.0113			
3	0.0206	0.0062	0.0103			
4	0.0173	0.0052	0.0086			
5				0.0350	0.0012	0.0070
6				0.0232	0.0008	0.0046
7				0.0123	0.0004	0.0025
8				0.0123	0.0004	0.0025
9				0.0122	0.0004	0.0024

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
Calculated mean dietary exposure to PCDD/F from the 6 typologies of infant formulae in the first 9 months of life (pg/kg bw per day)

Months	PCDD/F					
	Mf	Sf	HAf	fMf	fSf	fHAf
	WHO-TEQ/kg bw per day					
1	1.29	1.61	3.54			
2	1.12	1.41	3.09			
3	0.98	1.23	2.70			
4	0.80	1.00	2.20			
5				0.47	0.23	0.12
6				0.31	0.15	0.08
7				0.16	0.08	0.04
8				0.16	0.08	0.04
9				0.16	0.08	0.04

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

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