CONTAMINATION LEVELS OF DIOXINS, PCBs AND PBDEs IN FOODS FOR INFANTS AND YOUNG CHILDREN

Ceci R, Diletti G, Migliorati G, De Benedictis A, Pellicciotti S, Pirito L, Scortichini G*

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", via Campo Boario, 64100 Teramo

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), commonly referred to as dioxins, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are a group of halogenated aromatic compounds belonging to the list of persistent organic pollutants (POPs) under the Stockholm Convention[1].

They are ubiquitous and persistent contaminants of the global ecosystem, since they have been detected in almost every component of the biosphere, including air, sediments in aquatic and terrestrial ecosystem, fish, wildlife, domestic animals and humans.

These compounds are chemicals of concern because of their potential cancer and non-cancer health effects. Recent studies showed that human exposure to these contaminants is linked to reproductive disorders including decreased sperm count and delayed onset of puberty, developmental disorders such as foetal malformations, neurological effects and at high doses cancer[2-7].

Human exposure to these contaminants can occur from a variety of routes, including consumption of contaminated food, with products of animal origin and fish making the greatest contribution to this exposure.

In this context infants and young children can have a relatively high intake of foods relative to their body weight, and then they would be exposed, as a group, to higher concentration of toxic contaminants than adults.

Aim of this study was to evaluate the levels of PCDD/Fs, dioxin-like PCBs (DL-PCBs), non-dioxin-like PCBs (NDL-PCBs) and PBDEs in a number of foods for infants and young children purchased on the retail market.

Materials and methods

<u>Sampling</u>

Food samples were selected to meet the nutritional needs of infants from birth to 3 years of age and for each food category different trade marks were considered, taking into account their presence on the market.

A total of 54 samples were randomly collected between 2008 and 2009 from supermarkets and pharmacies in Teramo (Italy). Details of the different food categories such as the number of samples are given in Table 1.

Sampling included dry powdered infant formulae that are intended to be reconstituted with water, milk for young children, and meat and fish based baby foods sold in jars, ready to eat.

The dry baby foods included two typologies of infant formulae, "starting" and "follow-on" formulae.

Infant formulae samples were stored at room temperature while milk for young children, meat and fish based baby food samples were stored at + 4°C until analysis.

<u>Chemical analysis</u>

Samples were homogenized and tested by validated methods routinely used for 17 PCDD/Fs, 12 DL-PCBs, 6 NDL-PCBs (PCB # 28, 52, 101, 138, 153, 180) and 9 PBDEs (PBDE # 28, 47, 66, 85, 99, 100, 153, 154, 183) congeners monitoring in food and feed and successfully verified in a number of interlaboratory studies.

Before analysis all samples were spiked with the specific PCDD/Fs, PCBs and PBDEs standard solutions, a mixture of $^{13}C_{12}$-labelled congeners (Wellington Laboratories, Ontario, Canada). All samples (except milk and infant formulae) were extracted by accelerated solvent extraction (ASE) using an ASE 300 (Dionex, California, USA) instrument with a mixture of n-hexane and acetone 80:20 (v/v). An ethyl alcohol and ammonia solution was first added to the milk samples and reconstituted infant formulae, and then fat was extracted by a mixture of diethyl ether and petroleum ether 1:1 (v/v). After solvent evaporation, gravimetric lipid determination was performed.

The extracted fat was dissolved in hexane and the clean-up procedure was carried out in three steps. First a double liquid-liquid partitioning process with sulphuric acid was performed to remove the lipid component. Then the extract was dissolved in hexane and purified on a multilayer silica column (7 g of silica gel and acidic

silica) to separate PCDD/Fs and PCBs from PBDEs. In this step, the fraction containing PCDD/Fs and PCBs was eluted with hexane while the fraction containing PBDEs was eluted with dichloromethane/hexane 10:90 (v/v). Finally the fraction containing PCDD/Fs and PCBs was purified by means of an automated clean-up process with a Power-Prep® system (Fluid Management System, Massachusetts, USA) using disposable columns (multilayer silica, alumina and carbon). The fraction containing PCBs was collected after elution from the alumina column, while the fraction containing PCDD/Fs was eluted from the carbon column.
The three fractions were concentrated, first under vacuum and then under nitrogen stream and the remainders were dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labelled congeners).
A laboratory blank and a control sample were analysed for each batch of five and ten samples, respectively.
PCDD/Fs and PBDEs were analysed by gas chromatography (GC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.10 μm film thickness (J&W Scientific, California, USA) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10000 operating with electron ionisation (EI) at 40 eV, in the selected ion monitoring (SIM) mode. The GC-HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XP (Thermo Fisher, Bremen, Germany). PCBs were separated by HRGC on a VF-5 MS capillary column (60 m x 0.25 mm, 0.25 μm film thickness (Varian, California, USA) and determined by HRMS, in the same operating conditions adopted for PCDD/Fs.
Toxic equivalent (WHO-TEQ_{98} and WHO-TEQ_{05}) values for PCDD/Fs and DL-PCBs were calculated using the toxic equivalent factor model proposed by the World Health Organization in 1998[8] and then revised in 2005[9]. To allow comparison with papers published after the official adoption of the new TEFs, the WHO-TEQ_{05} was calculated.
WHO-TEQ values were calculated as upper bound concentrations assuming that all values of specific dioxins congeners below the limit of determination (LOQ) are equal to the respective LOQ.

Results and discussion
Results of chemical analysis of PCDD/Fs and DL-PCBs, expressed as WHO-TEQ_{98} and WHO-TEQ_{05} on a whole weight basis (pg WHO-TEQ/g product), are shown in Table 2, while the concentrations of NDL-PCBs and PBDEs, expressed as sum of the congeners analysed (pg/g product) are reported in Table 3.
To compare the contamination levels of PCDD/Fs and DL-PCBs with literature data only the WHO-TEQ_{98} values were considered. The concentration levels were relatively low for all groups of contaminants. In fact a high proportion of congeners were at concentrations next to or below their respective LOQs, especially in dry powdered infant formulae and milk for young children.

Meat and fish based baby food
PCDD/Fs levels ranged between 0.002 and 0.059 pg WHO-TEQ_{98}/g and there were no significant differences in mean contamination levels for the two categories of food. These data are in the same range detected in a Swedish study[10] (0.009-0.068 pg WHO-TEQ_{98}/g) and below the results obtained in the United States[11] (0.08-0.155 pg WHO-TEQ_{98}/g) and Japan[12] (<0.001-0.135 pg WHO-TEQ_{98}/g).
For meat based baby food, the lowest PCDD/Fs mean concentration was measured for pork samples (0.003 pg WHO-TEQ_{98}/g), while the highest level was obtained for horse meat samples (0.042 pg WHO-TEQ_{98}/g). The remaining three types of foods (beef, lamb and chicken) recorded similar contamination levels. Among fish based baby food, the salmon showed the highest PCDD/Fs mean level (0.024 pg WHO-TEQ_{98}/g), while sea-bream and hake samples showed the lowest contamination levels (0.005 and 0.006 pg WHO-TEQ_{98}/g, respectively). These findings are comparable with the data reported in a recent survey carried out in Spain[13] that showed PCDD/Fs values in meat slightly higher than those found in fish.
DL-PCBs levels ranged between 0.001 and 0.115 pg WHO-TEQ_{98}/g and fish samples were more contaminated than meat samples. Among the meat based baby food, the highest DL-PCBs concentrations were measured in horse meat samples with a mean value up to ten times higher than the mean levels detected in all the others meat based samples. For fish based baby food, the highest DL-PCBs mean concentration was measured in salmon samples, while the lowest level was found in hake samples.
The NDL-PCBs levels, expressed as sum of six congeners, ranged between 25.2 and 1300 pg/g. In this study, the contamination levels detected in fish samples were higher than those found in meat samples. These data are in disagreement with the results obtained by Lorán et al. (2010) that found NDL-PCBs levels in meat five times

higher with respect to fish samples. Among all food samples, horse and salmon were the most contaminated with a mean value of 215 and 1077 pg/g, respectively.

Contamination levels of PBDEs, expressed as sum of nine congeners, ranged between 22.6 and 247 pg/g. Fish samples showed a mean contamination two times higher than that for meat samples. Moreover, salmon was the most contaminated food with a mean value of 204 pg/g. PBDEs levels were in the same range for all meat samples except for a lamb meat sample at a concentration of 132 pg/g.

Literature on the levels of PBDEs in baby food is rather scarce. Moreover, the comparison with published data is difficult due to the high proportion of non detected congeners. In particular, the contamination levels recorded in this study are below those reported in a survey carried out in 2008 in the United States[14] as well as those encountered in eleven European countries, according to a recent report of the European Food Safety Authority[2].

Dry powdered infant formulae and milk for young children

PCDD/Fs levels ranged between 0.002 and 0.100 pg WHO-TEQ_{98}/g. Contamination levels were very low except for a powdered milk sample that showed a concentration of 0.100 pg WHO-TEQ_{98}/g. Excluding this value, the contamination varied from 0.002 to 0.047 pg WHO-TEQ_{98}/g. These levels are below the data reported in a recent study[15], where 42 different infant products, representative of the main brands marketed in Europe, were sampled in 6 European countries. The mean value calculated from the European survey was 0.04 pg WHO-TEQ_{98}/g, for both starting and follow-on formulae.

DL-PCBs levels ranged between 0.001 and 0.018 pg WHO-TEQ_{98}/g and these values are higher than those reported in the above cited study[15] (0.001-0.003 pg WHO-TEQ_{98}/g).

Contamination levels of NDL-PCBs were in the interval 6.95-154 pg/g. Dry powdered infant formulae showed higher contamination levels with respect to milk for young children. These results are in the same range as or below to the levels reported in a study conducted Spain in 2009[16].

Regarding PBDEs levels, all congeners were below the LOQ in all samples.

In conclusion, the contamination levels of the substances taken into account are comparable to the levels reported in the literature and they do not raise safety concern. The particular vulnerability of the population group under consideration makes desirable the implementation of chemical contaminants monitoring programs in baby foods, in order to ensure a consistent high level of safety against risks caused by those substances and to assess time trends in contamination levels, with the final aim of their progressive reduction.

Acknowledgements

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Table 1. Different food categories and samples analysed

Food categories	Species	N. samples
Dry powdered infant formulae	--	19
Milk for young children	--	5
Meat based baby food	Lamb	3
	Chicken	5
	Beef	4
	Pork	4
	Horse	3
Fish based baby food	Sea-bream	2
	Salmon	2
	Sea-bass	2
	Trout	3
	Hake	2

Table 2. Mean PCDD/Fs and DL-PCBs levels in foods for infants and young children, expressed as WHO-TEQ_{98} and WHO-TEQ_{05} (in brackets minimum and maximum values are reported)

Food categories	PCDD/Fs (pg WHO-TEQ_{98}/g)	DL-PCBs (pg WHO-TEQ_{98}/g)	PCDD/Fs (pg WHO-TEQ_{05}/g)	DL-PCBs (pg WHO-TEQ_{05}/g)
Dry powdered infant formulae	0.014 (0.004-0.100)	0.008 (0.002-0.018)	0.013 (0.004-0.084)	0.006 (0.001-0.018)
Milk for young children	0.003 (0.002-0.004)	0.006 (0.001-0.015)	0.003 (0.002-0.003)	0.005 (0.001-0.014)
Meat based baby food	0.013 (0.002-0.059)	0.020 (0.001-0.105)	0.011 (0.002-0.051)	0.019 (0.001-0.100)
Fish based baby food	0.011 (0.002-0.030)	0.058 (0.005-0.115)	0.009 (0.002-0.023)	0.044 (0.004-0.079)

Table 3. Mean NDL-PCBs and PBDEs levels in foods for infants and young children, expressed as analytical sum of analysed congeners (in brackets minimum and maximum values are reported)

Food categories	NDL-PCBs (pg/g)	PBDEs (pg/g)
Dry powdered infant formulae	88.0 (30.8-154)	45.5 (*)
Milk for young children	26.1 (6.95-59.6)	6.45 (*)
Meat based baby food	89.8 (25.2-224)	39.8 (22.6-132)
Fish based baby food	525 (39.9-1303)	92.2 (39.1-247)

(*) All congeners were below the corresponding LOQ