

## Determination of polychlorinated biphenyls in milk based infant formulas

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### Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous highly toxic compounds distributed in environment. Although their use and production have been banned for decades, residues can still be found in foods due to their lipophilicity, persistence, and ample use in the past in a wide scale of applications<sup>(1-3)</sup>.

Mixtures of the non-dioxin-like PCBs are generally assessed on the basis of a chemical analysis of the seven so-called "indicator PCBs", these were selected as suitable representatives for all PCBs because they are predominantly present in biotic and abiotic matrices<sup>1</sup>. The IUPAC numbers of these indicator PCBs are 28, 52, 101, 118, 138, 153 and 180.

Although WHO recommends breast feeding as the feeding choice for babies, infant formulas are an alternative to breast-milk. However, information about the presence of PCBs in this kind of food is scarce. The aim of the present work was to estimate the extent of PCB contamination in breast milk substitutes collected in Spain.

### Methods and Materials

Samples of infant formulas were acquired from local markets, big supermarkets and chemist. The 10 different trades studied were selected among the best well-known in Spain. Totally, 70 samples divided in three groups were analyzed, 25 infant formulas, 25 follow-on formulas and 20 special lactose-free formulas. A composite (1 kg) was made by mixing proportional pooled samples of 5 different batches from the same commercial trade. Lastly, 14 analytical samples were made up, 5 of infant formula, 5 of follow-on formula and 4 of special lactose-free formulas

These commercial infant foods contain skimmed cow milk and mixtures of vegetable oils that are added to these products in order to reach the nutrient requirements for children at this age. Infant formulas are those for children from 0 to 6 months of age and follow-on formulas are for infants 6 months and older. In addition, lactose-free formulas are specially adapted to children with lactose intolerance.

After homogenisation, ~20g of sample was weighed for extraction with an acetone/hexane mixture (1:1). The extracted lipid content was determined gravimetrically after evaporation of the solvents. An aliquot of 0.5g of the extracted fat was dissolved in hexane and purified on alumina chromatographic column. The eluting fraction containing the compounds of interest was collected and concentrated. Solvents employed were of Pestiscan grade supplied by Lab-Scan (Dublin, Ireland). Identification and quantification of the seven indicator PCBs was performed by injection of the concentrated eluates into a high resolution gas chromatography system (HRGC) coupled to Ion Trap MS/MS (Varian, Saturn 2000 MS/MS). Chromatographic separation was achieved with a DB-5 (Varian Factor Four) fused-silica capillary column (60m x 0.25 mm ID, 0.25 µm film thickness) with helium as the carrier gas at a linear velocity of 1 ml/min in the splitless injection mode (2µl).

A control sample of fortified infant formulas were inserted in each batch of samples to verify the accuracy and precision of the measurements. Blank samples were also included in the quality control analysis.

Quantification limits for the different PCB congeners were set in 0.5 ng/ml. The concentration of the non-detected congeners were calculated as the LOD.

### Results and Discussion

The concentrations of PCBs in foodstuffs collected are given in Table 1. Results are presented for the seven

indicator congeners, the sum of all of them is also shown. The average level in ng/g fat was below 1 ng/g fat in the three different formulas studied. The most variability between different brands was found in follow-on formulas. Generally, the average concentration of PCBs gets lower as the chlorination grade goes up from trichloro to heptachloro substituted congeners.

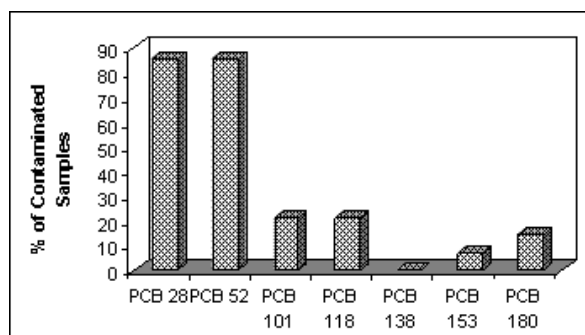
The sum of PCBs was higher in follow-on products than in those intended for infants from birth. This difference has been previously found for *ortho* (indicators)PCBs by the UK government<sup>2</sup> and it's in concern with the different nutrients content.

**Table 1.** Average levels of PCBs in infant formulas.

PCB congener	Mean $\pm$ SD (ng / g fat)		
	Initial Formula n= 5	Follow-on formula n = 5	Lactosa free-formula n = 4
PCB-28	0.18 $\pm$ 0.14	0.53 $\pm$ 0.66	0.35 $\pm$ 0.36
PCB-52	0.06 $\pm$ 0.04	0.10 $\pm$ 0.08	0.06 $\pm$ 0.05
PCB-101	0.03 $\pm$ 0.01	0.03 $\pm$ 0.03	0.02 $\pm$ 0.06
PCB-118	0.02 $\pm$ 0.01	0.04 $\pm$ 0.04	0.02 $\pm$ 0.007
PCB-138	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.007
PCB-153	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02	0.02 $\pm$ 0.007
PCB-180	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02	0.02 $\pm$ 0.008
$\Sigma$ PCBs	<b>0,35 <math>\pm</math> 0,15</b>	<b>0,76 <math>\pm</math> 0,82</b>	<b>0,50 <math>\pm</math> 0,43</b>

All samples analyzed were found contaminated at quantifiable levels with almost one PCB. Congeners 28 and 52 were the most detected (86% of samples studied) while PCB 138 was below the limit of detection in all samples studied as shown in figure 1.

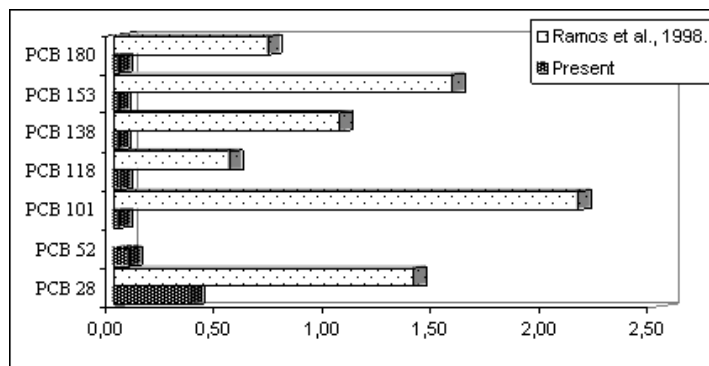
**Figure 1.** Presence of individual PCB congeners in infant formulas.



In general, the concentrations of PCBs observed in this study were comparable with those reported for Schechter et al.<sup>4</sup>, who analysed PCBs 28, 138, 153 and 180 in one soybean infant formula and found concentrations below detection limits (2 ng g<sup>-1</sup> of lipids). However, these values are below of those calculated by Ramos et al.<sup>3</sup> in 1998 in soybean infant formulas collected in Spain as it is shown in figure 2.

In any case, levels of PCBs in infant formulas seem to be below of those found in milk by Baars et al.<sup>5</sup>, in The Netherlands of 4 ng g<sup>-1</sup> fat and far below of those found in breast milk by Glynn et al.<sup>6</sup>, for the sum of 6 indicators PCBs: 149,81 ng/g fat and by Wang et al.<sup>7</sup> for the sum of 7 markers PCBs: 55.42 ng/g fat. This is consistent with Lackmann et al.<sup>8</sup>, who found higher serum concentrations of organochlorine compounds in breast-fed than in bottle-fed infants.

**Figure 2.** Comparative values for PCBs found by Ramos et al., (1998) in soybean infant formulas and those calculated in the present study.



These studies are in agreement with the observed decrease in the presence of the seven indicator PCBs, in food. Since no tolerance limit has been set for PCBs in foods by the EU or the Spanish Government their toxicological evaluation becomes more difficult. In any case the low values of PCBs measured in infant formulas are not supposed to be a risk in nursing infants through the consumption of this kind of substituted breast milk.

Although consumption of infant formulas is not supposed to be a risk with respect to the low levels of PCBs found in this study, Health Departments, WHO and the UK FSA encourage breastfeeding on the basis of the convincing evidence of the overall health and development of the infant<sup>2</sup>. This means that breast milk is better than infant formulas for feeding infants.

### Acknowledgment

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### References

1. ATSDR, 2000. Toxicological Profile for Polychlorinated Biphenyls (Update). Agency for Toxic Substances and Disease Registry, US Public Health Service, Atlanta, GA, USA.
2. Food Standards Agency UK (2004). Dioxin and dioxin-like PCBs in infant formulae. Food Survey Information Sheet 49/04.
3. Ramos L., Torre, M., Laborda, F., Marina M.L. (1998) Determination of polychlorinated biphenyls in soybean infant formulas by gas chromatography. *Journal of Chromatography A*, 823, 365-372.
4. Schecter, A., Fürst, P., Fürst, Ch., Meemken, H., Groebel, W., Vu D.Q. (1989). Levels of polychlorinated dibenzodioxins and dibenzofurans in cow's milk and in soy bean derived infant formulas sold in the United States. *Chemosphere* 19, 913-918.
5. Baars, A.J., Bakker, M.I., Baumann, R.A., Boon, P.E., Freijer, J.I., Hoogenboom, L.A.P., Hoogerbrugge, R., van Klaveren, J.D., Liem, A.K.D., Traag, W.A., de Vries J. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands. *Toxicological Letters* 151, 51-61.
6. Glynn, A.W., Atuma, S., Aune, M., Darnerud, P.O., Cnattingius, S. (2001) Polychlorinated Biphenyl Congeners as

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Markers of Toxic Equivalents of Polychlorinated Biphenyls, Dibenzop-dioxins and Dibenzofurans in Breast Milk. Environmental Research Section A 86, 217-228.

7. Wang, S.-L., Lin, C.-Y., Guo, Y.L., Lin, L.-Y., Chou, W.-L., Chang, L.W. (2004) Infant exposure to polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs)-correlation between prenatal and postnatal exposure. Chemosphere 54, 1459-1473.

8. Lackmann, G.-M., Schaller, K.H., Angerer, J. (2004). Organochlorine compounds in breast-fed vs. Bottle-fed infants: preliminary results at six weeks of age. Science of the Total Environment, 329, 289-293.

9. Baker, M.I., Baars, A.J., Baumann, R.A., Boon, P.E., Hoogerbrugge, R. (2003) RIVM I report 639102025/2003, RIKILT report 2003.014. Indicator PCBs in foodstuffs: occurrence and dietary intake in The Netherlands at the end of the 20<sup>th</sup> century.