Levels of polychlorinated biphenyls (PCBs) in whole milk powder and estimated daily intake for a population of children

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ABSTRACT: Polychlorinated biphenyls (PCBs) are chemical contaminants classified as persistent organic pollutants. Although, their use has been banned for several decades, PCBs are still scattered in the environment and therefore, all living organisms may be exposed to these compounds. Diet, especially fatty foods such as milk, has been recognized as one of the main sources of human exposure to PCBs. Hence, the aim of this study was to evaluate the residual levels of indicator PCBs in whole milk powder consumed by preschool children in the Municipality of Imbé, State of Rio Grande do Sul, Brazil and to determine the estimated daily intake of these PCBs through this food. Analyses were performed by GC-µECD and the results were confirmed by GC/MS. The PCBs 28, 52 and 153 displayed values below the limit of quantification. The PCBs 138 and 180 showed mean values of 0.073 and 0.157ng.g⁻¹ lipid, respectively. These values were below the reference limits established by the European Community. The estimated total daily intake of PCBs was 0.110ng.g⁻¹ lipid of body weight per day, a value lower than that established by the legislations of Belgium and Norway.

Key words: indicator PCBs, milk powder, daily intake, children.

INTRODUCTION

Polychlorinated biphenyls (PCBs) belong to the group of chemical contaminants classified as persistent organic pollutants (POPs), due to their persistence and bioaccumulation power in biotic organisms and abiotic systems (KABIR et al., 2015). The term “PCBs” refers to a family of compounds including 209 congeners, which are divided into two main groups: “dioxin-like PCBs (DL-PCBs)” and “non-dioxin-like PCBs (NDL-PCBs)”. The second group, NDL-PCBs or “indicator PCBs”, is composed of six congeners (PCB 28, 52, 101, 138, 153 and 180), which are generally used as markers in pollution studies (AHMADKHANIHA et al., 2017).

Due to several physical-chemical properties, such as low flammability, stability to high temperatures and electric currents, PCBs had been widely produced and used for decades throughout...
the world, mainly as hydraulic and dielectric fluids in transformers and capacitors (KABIR et al., 2015). In spite of their many industrially relevant properties, they are compounds of difficult elimination and degradation, causing accumulation of toxic waste in water, animals, and food (FILLMANN et al., 2002; FOCANT et al., 2002).

According to the International Agency for Research on Cancer (IARC), PCBs are classified as carcinogenic to humans (Group 1). Due to their serious effects on health and the environment, their processing and distribution have been banned in almost all industrialized countries since the late 1980s (EFSA, 2010). Nevertheless, PCBs are still scattered in the environment due to leakage of old capacitors and transformers (ATSDR, 1990). In Brazil, the prohibition dates back to 1981, but their use is still allowed in old electro-electronic equipment until these are replaced with PCB-free products (SCHWANZ et al., 2012).

Therefore, since PCBs are still present in the environment (BÁNYIOVÁ et al., 2017), living organisms, in general, are exposed to these compounds both directly, through contamination of air, sediments, and water, and indirectly (SCHWANZ et al., 2012). Diet has been widely recognized as the main indirect source of ingestion of toxic products such as PCBs. COCCO et al. (2015) demonstrated the presence of indicator PCBs residues in rice and bean, both foods of plant origin, of Rio Grande do Sul State (Brazil). However, in general, fatty foods of animal origin are the main sources of human exposure to PCBs, which are lipophilic and have the propensity to bioaccumulate in biota (FADAEI et al., 2015; GILBERT et al., 2015). Thus, milk fat is probably one of the main food products potentially responsible for human exposure to PCBs and the consumption of milk and dairy products can significantly contribute to the dietary intake of these compounds.

Milk and dairy products, in particular, have received special interest since they are widely consumed by children, as they are rich in several nutrients that are essential for growth and maintenance of a healthy life (MOSCHONIS et al., 2016) and also provide immunological protection (LARSSON et al., 2015; LAMARCHE et al., 2016). Few studies have investigated the presence of PCBs residues in milk samples. BELLA et al. (2014) analyzed the presence of organochlorine pesticides (OCPs) and PCBs (DL-PCBs and indicator PCBs) in 45 samples of donkey milk from conventional Italian farms. In Brazil, HECK et al. (2007), was the first and still unique study to analyze the presence of residues of OCPs and indicator PCBs (congeners 10, 28, 52, 138 and 180) in raw, pasteurized, and UHT milk from Rio Grande do Sul State. In both studies, the contaminants did not exceed the maximum residues levels (MRLs) for PCBs in milk according to the European Community.

Due to the scarcity of experimental data on this type of food, additional research is needed to investigate the levels of PCBs residues in milk and assess the health risk related to its consumption by susceptible populations, such as children.

The aim of this study was to evaluate the residual levels of indicator PCBs in whole milk powder consumed by preschool children in the Municipality of Imbé, State of Rio Grande do Sul, Brazil and, in addition, to determine the estimated daily intake of these PCBs through milk consumption.

**MATERIALS AND METHODS**

A total number of 38 whole milk powder samples (Brand A) were analyzed. These samples were obtained from feeding for preschool children in the municipal early childhood education schools located in the Municipality of Imbé, Rio Grande do Sul, Brazil.

Chromatographic grade hexane and magnesium silicate (60-100 mesh) were acquired from Mallinckrodt Baker® (Phillipsburg, USA). Magnesium silicate was previously activated at 150°C for 12h, deactivated with the addition of 2% double-distilled water, and shaken prior to use. Other reagents used, such as petroleum ether and sodium sulfate, were acquired from Merck® (Darmstadt, Germany) and Vetec® (Rio de Janeiro, Brazil), respectively.

The analytical standards of the polychlorinated biphenyls (PCB 28, 2, 4, 4'-trichlorobiphenyl; PCB 52, 2, 2', 3, 5'-tetrachlorobiphenyl; PCB 153, 2, 2', 3, 4, 4', 5'-hexachlorobiphenyl; PCB 138, 2, 2', 3, 4, 4', 5'-hexachlorobiphenyl; PCB 180, 2, 2', 3, 4, 4', 5, 5'-heptachlorobiphenyl) were acquired from SUPELCO, Inc., Bellefonte, Pennsylvania (USA), with a degree of purity of more than 99%.

Residues of PCBs were determined in the fat extracted from the milk powder samples. The procedure for fat extraction and purification was based on the method described by SANDMEYER (1992), with some adaptations: 55g of powdered milk were reconstituted with 110ml of distilled
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The PCBs were extracted by following the methodology of MARTINEZ et al. (1997), in which 0.1g of the fat sample was added to 1ml of n-hexane and vortexed until full dilution. Next, the sample was loaded on a chromatographic column containing 10g of magnesium silicate and anhydrous sodium sulfate, and eluted with 80mL of n-hexane for the extraction of PCBs. The eluted solvent was filtered using cotton plus sodium sulfate and rotary evaporated to full dryness. Subsequently, it was stored at -20°C until chromatographic analysis. Next, the sample was reconstituted with 1mL of n-hexane. For each set of extracted samples, a blank was made to check for possible contaminations during the analytical procedure.

Residues of PCBs were analyzed by using an Agilent gas chromatograph, model 6890N, equipped with a Ni^{60} electron capture micro detector (GC-μECD) and an automatic injector HP 7683. Separation of the analytes was performed on a capillary column fused silica DB-1701, (length: 30m; internal diameter: 0.25mm; stationary phase thickness: 0.25μm). Helium was used as carrier gas under a constant pressure of 18.29psi, providing a flow rate of 1.5mL/min. The initial oven temperature was set at 60°C (2min), followed by a heating ramp of 5°C/min until 220°C (5min) and, finally, the temperature rose to 300°C at a rate of 20°C/min (2min). The detector operated in the selected ion monitoring mode (SIM) with electron ionization (EI) at 70 eV.

Basic parameters for the analyses of residues were validated: linearity, precision, recovery, accuracy, limit of detection, and limit of quantification. For the linearity study, a series of five samples with the addition of increasing concentrations of standards (from 0.25 to 40ng. mL⁻¹) were prepared. These samples were prepared in triplicate, on different days, and injected in duplicate in the chromatographic system. Additionally, for each triplicate, two negative control samples were prepared, one without sample and the other without matrix. The obtained data were used to create a calibration curve for each of the evaluated compounds.

The precision was assessed by using the relative standard deviation (RSD) estimate, also known as coefficient of variation (CV), which was lower than 18%, indicating the effectiveness of the method for repeatability (intra-day) and for intermediate accuracy (inter-day). For repeatability, 5 equal samples were prepared, all contaminated with 8ng.mL⁻¹ of each of the compounds under examination. These samples were analyzed according to the proposed method, from extraction (all on the same day) until the chromatographic analysis. For the accuracy study, the standard addition technique was used, consisting of adding different known quantities of certified standards of the analyte of interest into the matrix, prior to the preparation of the sample. For each compound, four samples were prepared by the addition of standard at the concentrations of 0, 8, 12 and 20ng. mL⁻¹, respectively, and analyzed in triplicate, and the measured quantities were related to the added amounts. The limits of detection (LOD) and of quantification (LOQ) were evaluated using the average blank values method.

In order to estimate the extent of human exposure to chemicals in food, three essential data are required: the concentration of the substance in the food (ng.kg⁻¹), the food consumption (kg) and the body weight (kg) of the studied individuals or population. The estimate of daily intake (EDI) or presumed exposure can be defined, in general, by the equation below.

\[
\text{EDI} = \frac{\text{C} \times \text{F} \times \text{B}}{1000}
\]

where C is the concentration of the substance in the food, F is the food consumption (kg), and B is the body weight (kg) of the studied individuals or population.

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EDI = Concentration of the compound X food consumption

Body weight

Data for the preschool students (weight and total milk powder consumption per capita) were collected in compliance with the guidelines and standards on research involving human subjects, presented in Resolution 196/96 of the National Health Council (BRASIL, 1996) and pertaining to data confidentiality, as well as privacy and integrity of participants. The Department of Education was requested to authorize the research. The study was approved by the Ethics Committee of the Universidade Federal de Santa Maria under the Certificate of Presentation for Ethical Assessment number 0100.0243.000-10. Size of the population sample was calculated, according to BARBETTA (1999), considering a tolerable sampling error of 5%. The sample consisted of 236 children (113 girls and 123 boys), with age between 24 and 78 months (average: 47 months). The data on milk consumption and weight of the population exposed to PCBs through the consumption of whole milk powder by preschools in the Municipality of Imbé/RS were obtained from SANTOS et al. (2015).

The estimated dietary exposure to PCBs, calculated from the amount of residues reported in milk powder, milk consumption per day, and children’s weight, was compared to the Tolerable Daily Intake (TDI) adopted by countries such as Belgium and Norway. The TDI is defined as the maximum amount of a potentially toxic substance that can be consumed by humans without endangering their health in the long term (CIMENCI et al., 2013). Results obtained in the research were analyzed by the Statistica® 7.0 software. The average concentrations and the incidence of PCB congeners in whole milk powder were calculated.

RESULTS AND DISCUSSION

Table 1 summarizes the analytical parameters of the method employed for PCB analysis. The analytical characteristics were considered satisfactory for the aims of the analysis.

Samples were analyzed according to the relevant methodology, and the results represent the arithmetic mean, considering n=38 (for all samples) and n=4 (for the samples containing PCBs in concentrations higher than the LOQ).

Table 2 shows the average concentrations, the incidence of PCBs, and the dispersion interval of the samples. The values are given in ng.g⁻¹ fat. From the 38 samples of milk powder analyzed, residues of PCBs 138 and 180 were detected in 7.9% of the samples, whereas PCBs 28, 52 and 153 showed values below the limit of quantification (<LOQ). The examination of whole milk powder showed a higher average concentration of PCB 180 when compared to PCB 138. In the study by SIROT et al. (2012), the PCB congeners n°52, 101, 138, 153, and 180, indicating environmental contamination, were detected in all liquid and solid samples, including milk powder.

Table 3 shows comparison between the average concentrations of PCB congeners in samples of whole milk powder (this study and RAMOS et al., 1998), sterilized (UHT), pasteurized, and raw milk (HECK et al., 2007). The average concentrations of PCBs 28, 52, 153, 138, and 180 reported in the present study were lower than those reported by RAMOS et al. (1998), in Spain. These authors analyzed three brands of whole milk powder and found detectable concentrations of PCBs 153, 138, and 180 in all of them. The authors did not evaluate the congeners 28 and 52, but analyzed other indicators of environmental contamination (PCBs 101 and 118) that were not evaluated in this study. The average values obtained in the present analysis of whole milk powder (Brand A) were also lower when compared to those reported by another study carried out in Rio Grande do Sul, in which HECK et al. (2007) analyzed sterilized, pasteurized, and raw milk samples. It should be pointed out that PCB 180 was approximately 75 times higher in pasteurized milk than in whole milk powder. The concentration of PCB 28 was be below the LOQ in both sterilized and powdered milk. However, PCB 138 was present in concentrations that were

| Parameter | PCB |
|-----------|-----|
| Linear range of concentration (ng.mL⁻¹) | 0.25 – 40 |
| $R^2$ | 0.9911 – 0.9978 |
| LOD (ng.g⁻¹) | 0.25 – 1.0 |
| LOQ (ng.g⁻¹) | 0.5 – 3.0 |
| Recovery (%) | 84.05 – 105.80 |
| Accuracy (DSR%) | 2.36 – 4.79 |
| Repeatability of the peak area (DSR%) | 6.75 – 12.55 |
| Intra-day | 11.26 – 17.76 |

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below the limit of detection (LOD) in raw and sterilized milk, when compared to milk powder. Considering the processing temperatures for milk powder (170-250ºC), UHT milk (135-150ºC), and pasteurized milk (72-75ºC), lower concentrations of PCBs were associated with higher processing temperatures, as in the case with powdered milk. These results suggested that the higher the processing temperature the lower the concentration of the indicator PCBs in the food.

Reductions in the concentration of pesticides in foods after thermal processing have already been reported (ABOU-ARAB, 1999; BALINOV A et al., 2006; YANG, 2012). ABOU-ARAB (1999), upon thermal treatment of tomatoes, reported reductions of 71-82% in the concentration of organophosphate pesticides, and of 31-45% in that of organochlorines, compounds also classified as persistent organic pollutants. The author claimed that pesticides had been decomposed by heating. BONNECHERE et al. (2012) reported that spinach cooking in a microwave oven and sterilization decreased the concentrations of pesticides by 50-80% and 50-100%, respectively. Notably, the sum of PCBs (i.e., the sum of the detected average concentrations of compounds above the LOQ in each sample, n=38) in the whole powdered milk supplied to preschoolers in the municipality of Imbé/RS was considerably lower (0.230ng.g⁻¹ fat) than those reported by HECK et al. (2007) in sterilized, pasteurized and raw milk (Table 3).

When SHIN et al. (2015) studied DL-PCBs and NDL-PCBs congeners, they detected a total PCB level of 0.699ng.g⁻¹ in powdered milk. LEE et al. (2016), focusing on different food groups obtained between 2004 and 2012, observed a decrease in DL-

### Table 2 - Mean, standard deviation (SD), incidence, minimum (min) and maximum (max) concentrations of PCBs congeners in samples of whole milk powder (ng.g⁻¹ fat).

| Congeners | All samples (n=38) | Positive samples (n=4) |
|-----------|--------------------|------------------------|
|           | Mean±SD            | Incidence (%)          | Mean (SD) | Min - max |
| PCB 28    | 0.000              | 0.0                    | ND        | ND        |
| PCB 52    | 0.000              | 0.0                    | ND        | ND        |
| PCB 153   | 0.000              | 0.0                    | ND        | ND        |
| PCB 138   | 0.073±0.257        | 7.9                    | 0.931 (0.168) | 0.782-1.114 |
| PCB 180   | 0.157±0.571        | 7.9                    | 1.990 (0.758) | 1.454-2.857 |
| ∑ PCB     | 0.230              | 2.921                  |           |           |

*Congeners with values lower than LOQ were designated as zero. **Congeners with values higher than LOQ. ND=Not detected. ∑ PCB = Sum of mean congener concentrations detected with values higher than LOQ.

### Table 3 - Mean concentrations of PCBs congeners in samples of whole milk powder, sterilized, pasteurized, and raw milk (ng.g⁻¹ fat).

| Congeners | Whole milk powder | Fluid milk |
|-----------|-------------------|------------|
|           | Brand A* | Brand B* | Brand C* | Brand D* | Sterilized* | Pasteurized* | Raw* |
| PCB 28    | 0.000    | NE       | NE       | NE       | 0.00        | 0.29        | 0.32  |
| PCB 52    | 0.000    | NE       | NE       | NE       | 1.02        | 1.43        | 0.59  |
| PCB 153   | 0.000    | 1.60     | 2.74     | 1.59     | NE          | NE          | NE    |
| PCB 138   | 0.073    | 1.72     | 2.41     | 1.47     | 0.00        | 0.14        | 0.00  |
| PCB 180   | 0.157    | 0.80     | 1.78     | 0.81     | 1.00        | 11.70       | 2.50  |
| ∑ PCB     | 0.230    | 4.12     | 6.93     | 3.87     | 2.02        | 13.56       | 3.41  |

*Present study (Rio Grande do Sul, Brazil). **Ramos, Torre and Marina, 1998 (Asturias, Spain). *Heck et al., 2007 (Rio Grande do Sul, Brazil). NE=Not studied. ∑ PCB=Sum of mean congener concentrations detected with values greater than LOQ.
PCBs in dairy products such as sheep milk and milk powder. As to the sum of NDL-PCBs (congeners 28, 52, 101, 138, 153 and 180) in food stuffs such as raw milk, dairy products, and butterfat, the Commission of the European Communities established the value of 40ng.g⁻¹ fat as a reference limit according to Regulation (CE) Nº 1881/2006, as amended by Regulation (CE) Nº 1259/2011 (EUROPEAN COMMISSION, 2011). Thus, the values of 0.230ng.g⁻¹ fat and 2.921ng.g⁻¹ fat found in the current study in the whole milk powder samples are below the reference limit established by the European Community. Comparison of these data with Brazilian national values is not possible, because no MRL was established by the Brazilian Legislation for PCBs in milk.

The average consumption of whole milk powder by the studied population was 32g.day⁻¹, corresponding to 8.3g of fat. The average weight of the preschoolers was 17.35kg (10.2-35.3kg) (SANTOS et al., 2015). Based on the concentration of PCBs in the analyzed samples, the amount of powdered milk ingested, and the average weight of the children, the estimate of the daily intake of PCB congeners can be calculated (Table 4).

The highest EDI index was 1.397ng.g⁻¹ fat/body weight/day, from the sum of the average concentrations (2.921ng.g⁻¹ fat) of the positive samples (n=4). In principle, the EDI can be utilized to assess the exposure of the population by comparing it to the Admissible Daily Intake (ADI). However, since there are no established ADI values for the PCB indicators in the Brazilian legislation, in relation to milk powder consumption by the Brazilian population, the EDI was compared to the TDI defined in countries such as Belgium and Norway (10ng.kg⁻¹ of body weight per day) (CIMENCI et al., 2013). However, the European Food Safety Authority (2010) and the FAO/WHO Expert Committee on Food Additives have not established a TDI for NDL-PCBs and; therefore, the effects on public health of the exposure to these compounds remain of uncertain interpretation.

**CONCLUSION**

The measurement of PCBs, indicating environmental contamination, in whole milk powder samples showed that these compounds presented values below the reference limits established by the European Community and incorporated into the Brazilian Legislation. The compounds that contributed to the sum of PCBs were the most chlorinated congeners (PCBs 138 and 180). The estimated daily intake by preschool children in the Municipality of Imbé, for the analyzed polychlorinated biphenyls, was lower than the tolerable daily intake as adopted in countries such as Belgium and Norway. As the values of the compounds detected in the whole milk powder samples and the estimated daily intake were below the recommended values, it can be said that the whole milk powder consumed by preschool children does not represent a toxicological risk as far as our evaluation was concerned. However, it is worthy to note that, due to its long-term consumption, milk may ultimately favor a significant exposure to these compounds and, in combination with other sources, potentially cause a high risk of contamination. Therefore, more studies should be carried out to establish specific legislation for Brazil regarding the Reference Limits and values for Admissible Daily Intakes. It is also worth emphasizing the need to monitor the residues of PCBs as indicators of environmental contamination in milk, due to the great importance and wide consumption of this food by the Brazilian population, especially children.

| Congeners | All samples (n=38) | Positive samples (n=4) |
|-----------|--------------------|------------------------|
|           | Mean               | Minimum                | Mean             | Maximum |
| PCB 138   | 0.035              | 0.374                  | 0.445            | 0.533   |
| PCB 180   | 0.075              | 0.696                  | 0.952            | 1.367   |
| ΣPCB      | 0.110              | 1.397                  |                    |         |
DECLARATION OF CONFLICTING INTERESTS

The authors declare that there are no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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