ORGANOCHLORINE PESTICIDES (OCPs) AND POLYCHLORINATED BIPHENYLS (PCBs) IN BOVINE MILK FROM REGIONS WITH DIFFERENT ALTITUDES IN BRAZIL

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Introduction

The last few decades have been marked by technological advances, economic development and considerable increase in world population. These facts resulted in the need of increase of food production and consequently in the creation of chemical compounds capable of satisfying human necessities and overcoming the problems of food production and storage. Among these compounds there are two groups of synthetic chemicals that have been intensely used after the World War II, the organochlorine pesticides (OCPs) and the polychlorinated biphenyls (PCBs). They share notable properties as high stability against decomposition or degradation in the environment, low solubility in water and high solubility in hydrocarbon-like environments, such as the fatty material of living matter[1]. These compounds provided great benefits to humans until their toxic effects began to be evidenced and they were banned in Stockholm Convention in 2004[2]. Organochlorine pesticides had a very important role not only in food production but also in public health and were crucial in fighting against diseases such malaria. Polychlorinated biphenyls, which are not pesticides, found a wide variety of applications in modern society and were widely used as coolant fluids in power transformers and capacitors, as plasticizers, as deinking solvents, as heat fluids in machinery, as waterproofing agents, etc[1].

OCPs and PCBs persistently contaminate the environment once they are chemically stable with long biological half-life, which leads to high biomagnification in the food chain across a wide range of trophic levels. They enter the environment through many different ways and can be found in air, soils, sediments, natural water bodies, snow and animal fats. Thanks to restrictions and bans, the environmental concentration of these compounds is dropping substantially in the word[1]. It has been shown that such kind of pollutants can be found even in remote regions as a result of their long-range transport in the atmosphere, precipitation and progressive volatilization in relatively warm locations and subsequent condensation in coolest environments. Therefore, increased concentrations of these contaminants were found in high latitudes as well as at high altitudes, because of their relatively low average temperatures[3,4].

The accumulation of OCPs and PCBs in the soil leads to their accumulation in plants. Therefore, grazing cows accumulate OCPs and PCBs residues by eating contaminated food and grass. Because of their lipophilic properties, these chemicals are stored in fat cells and excreted basically by milk, which presents high levels of fat[5,6]. Consequently, milk consumption may represent an important route of human exposure to these contaminants. Despite this fact there are no data concerning raw cow milk contamination by OCPs and PCBs. This study aims to fulfill the lack of data of OCPs and PCBs concentrations found in raw cow milks collected in regions of different altitudes of Brazil.

Materials and Methods

Cow milk samples were collected from 16 dairy cattle farms located in areas with altitudes above 900m and 14 dairy cattle farms located in regions below 200m altitude from South and Southeastern of Brazil. Glass bottles extensively washed with distilled water, n-hexane and acetone were used. A total of 10 liters of raw cow milk were collected from refrigerated tanks of each milking farm and therefore, the samples represented a pool of all productive cows in the farms. Milks collected. Samples were immediately stored at -20 °C until subsequent sample processing and before chemical analysis, they were unfrozen and submitted to lyophilization for total water removal.

Dried milks were spiked with PCB 103, 198 standards and were extracted using Soxhlet extraction and a mixture of n-hexano:acetone (7:3, v/v) for 16 hours. All extracts were evaporated and the fatty amount was gravimetrically determined at constant weight. The fat was dissolved in 10 mL of n-hexane and transferred into a clean-up column containing 100 g of acid silica (54% silica + 46% H_2SO_4). The column was eluted with 400 mL of n-hexane, which was concentrated up to 1 mL, partitioned in a Florisil column and eluted with 40 mL of n-hexane and 40 mL of dichloromethane. The two fractions were evaporated using a gentle nitrogen flow and ressuspended in 500 µL of isooctane containing 500 µL of 2, 4, 5, 6-Tetrachloro-m-Xylene (TCMX) as internal standard.

Quality control and assurance measures for all analytes were similar. Sample extraction and purification were carried out in batches that included a full method blank that was used to compute limits of detection and was assessed for the presence of native contaminants and data were rejected if any concentrations detected were unacceptable. The analytical recovery rate (using PCB 103 and 198 as standards) in samples varied between 80% and 120%. The gas chromatography-ECD analysis of sample extracts was preceded by the analysis of a standard solution to all OCPs and PCBs detected in this work. All chromatograms were scrutinized for chromatographic peak shape and resolution.

All analysis were carried out using a Gas Chromatography GC-2010 with an automatic injector AOC-20i and ECD (Shimadzu, Japan) and a a (5% phenyl) methylpolysiloxane 60m column (Quadrex Co., USA). Standards and extracts were injected in the splitless mode at 180 °C. Hidrogen was used as gas carrier at a flow of 2 mL min^{-1} and nitrogen was employed as auxiliary gas (45 mL min^{-1}). The temperature of the ECD detector was 310 °C. All Solvents, Silica and Florisil® used in this study were of trace analysis quality and were purchased from Merck Co., Sigma Aldrich Co. or TediaBrazil. OCPs and PCBs standards were obtained from AccuStandard (New Havent, CT, USA).

Results and Discussion

Total concentrations of OCP given as $ng.g^{-1}$ fat basis (mean, median, ranges and number of positive samples) found in cow milk in the two studied regions are shown in Table 1. The samples collected above 900m altitude were grouped as Region A while the samples collected below 200m were grouped as Region B. Our results of OCP are lower than previously reported in other countries, but showed the same order of magnitude of those observed in milk collected in the Brazilian State of Southern Mato Grosso[5,7,8].

Figure 1 shows that the samples collected at the highest altitudes (Region A) presented higher concentrations of OCPs than samples of Region B, except for HCB and p,p´-DDE.

Table 1. Mean, median and range (minimum-maximum) concentrations of OCPs ($ng.g^{-1}$ fat) in milk samples from high altitude regions (Region A) and low altitudes regions (Region B) from Brazil.

Pesticide	Region A			Region B		
	Mean	Median (min-max)	Positive samples	Mean	Median (min-max)	Positive samples
ALDRIN	0.61	0.82 (0,32 - 0.87)	16	0.22	0.32 (0 - 0.47)	12
ISODRIN	3.01	0.99 (0.76 - 3.32)	16	-	-	0
DIELDRIN	7.48	11.31(2.93 -12.33)	16	2.11	1.49 (0.73 - 2.45)	13
ENDRIN	8.45	6.28 (4.49 - 14.57)	16	2.82	2.01 (0.38 - 5.56)	14
ENDOSULFAN	1.38	1.97 (0.53 - 2.67)	16	1.61	2.17 (0 - 6.13)	14
HEPTACHLOR	2.27	1.98 (0.80 - 2.84)	16	1.52	1.16 (0.86 - 3.37)	12
ΣHEP.EPOXIDE	11.08	15.57 (2.93 - 18,29)	16	3.2	3.37 (1.27 - 4.68)	14
METHOXYCHLOR	2.78	1.63 (0.43 - 4,89)	16	1.05	0.87 (0.45 - 0.51)	11
MIREX	4.52	3.14 (1.38 - 8,92)	16	0.43	0.32 (0 - 0.53)	14
HCB	1.51	2.48 (1.33 - 2,64)	16	2.48	1.62 (1.58 - 3.39)	10
alpha-HCH	1.82	1.93 (1.52 - 1.93)	16	1.12	0.79 (0.63 - 1.94)	14
delta-HCH	1.17	1.13 (1.05 – 1.43)	16	-	-	0
gamma-HCH (Lindane)	8.35	8.59 (7.19 – 9.89)	16	1.32	1.86 (0.46 - 2.62)	14
Σ CHLORDANE	8.27	7.46 (3.88 - 11.38)	16	3.13	2.56 (1.96 - 4.54)	12
o,p´-DDT	3.42	4.03 (3.22 - 4.53)	15	1.93	1.17 (0.77 - 2.54)	12

p,p´-DDT	6.47	3.57 (2.46 – 7.15)	16	1.14	3.08 (0.79 - 5.25)	14
o,p´-DDD	8.06	7.83 (6.61 - 10.74)	16	2.09	3.52 (1.07 - 5,87)	12
p,p´-DDD	2.87	1.95 (1.43 – 3.71)	14	0.77	0.64 (0.50 - 1.16)	10
o,p-´DDE	9.13	6.27 (4.58 – 12.56)	16	1.63	1.89 (0.94 - 2.94)	11
p,p´-DDE	3,17	2,16 (1,04 - 4,18)	8	3,56	2.97 (0.67 – 5.27)	11

Positive samples= number of samples above LOD

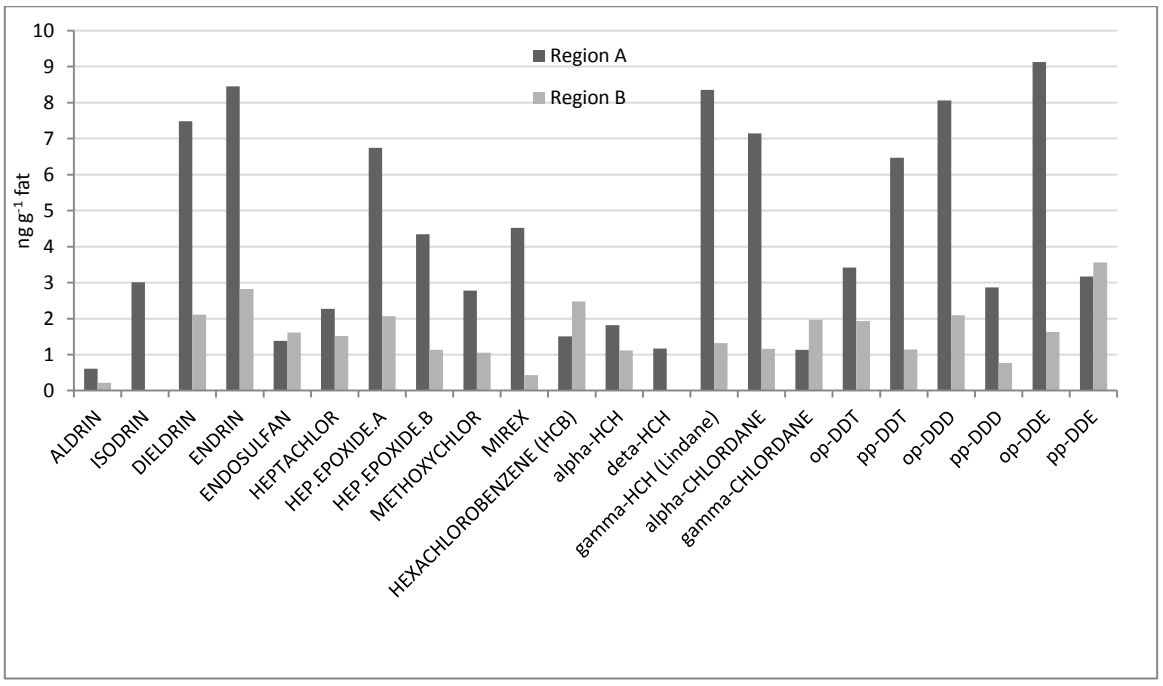

Figure1. OCP concentrations (ng g^{-1} fat) and profile in cow milks from regions with altitude above 900m (Region A) and regions below 200m of altitude (Region B).

Total PCBs level (ng g^{-1} fat) in cow's milk from regions above 900m altitude (Region A) and under 200m altitude (Region B) is given in table 2. Compounds with two, three and four chlorines were found in all samples and the highest level of PCB was found for those tetra-chlorinated ones (175.65 ng g^{-1} in Region A and 93.51 ng g^{-1} in Region B). .

Table 2. Mean ± Standard Deviation (SD) of PCBs (ng.g^{-1} fat) in milk samples from high altitude regions (Region A) and low altitude regions (Region B) from Brazil.

	Region A		**Region B**	
Congeners	**Mean ± SD**	**D**	**Mean ± SD**	**D**
Di-CB	14.87 ± 0,98	100%	10.46 ± 2,58	100%
Tri-CB	35.12 ± 2,46	100%	28.03 ± 3,31	100%
Tetra-CB	175.65 ± 0,78	100%	93.51 ± 9,55	100%
Penta-CB	80.56 ± 6,72	100%	38.05 ± 0,27	94%
Hexa-CB	36.89 ± 3,99	68%	22.09 ± 0,58	65%
Hepta-CB	29.38 ± 2,47	76%	6.05 ± 1,23	68%
Octa-CB	2.21 ± 0,23	70%	2.87 ± 0,34	71%
Nona-CB	9.67 ± 1,95	83%	0.41 ± 0,06	86%
Deca-CB	7.67 ± 1,49	63%	0.40 ± 0,03	79%
ΣPCB	392.02		201.87	

D* = percentage of samples above LOD

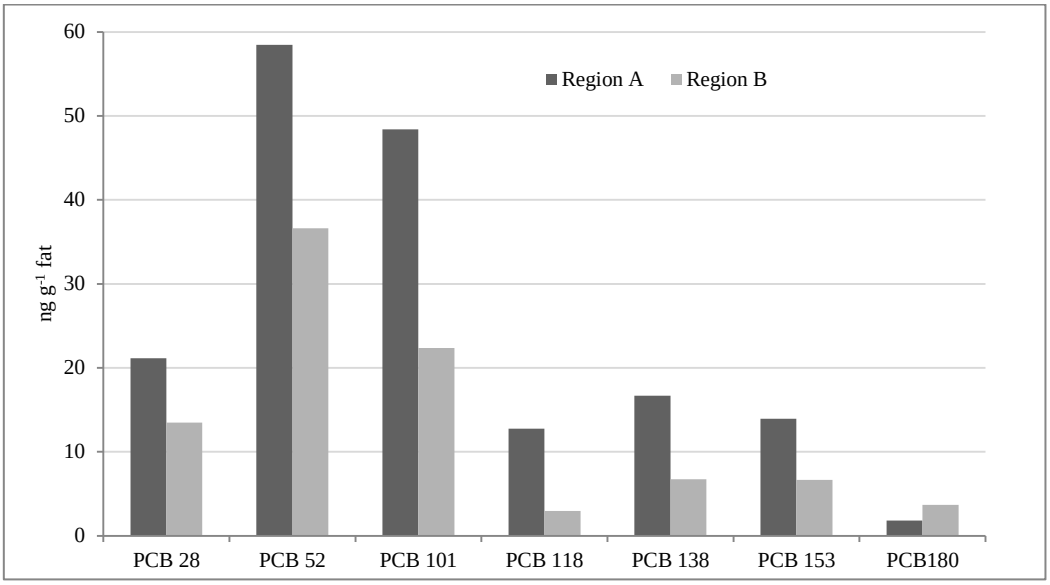

Figure 2. Level (ng g^{-1} fat) and profile of seven indicator polychlorinated biphenyls in cow's milk from regions with altitude above 900m (Region A) and regions under 200m of altitude (Region B).

Figure 2 show the profile of indicator PCBs in cow milk samples. Except for PCB 180, all PCB levels were higher in milks from Region A than from Region B. Our finding confirms that the sum of indicator PCBs is a suitable marker to assess the level of exposure of humans. Furthermore, this study demonstrates that, even in tropical or subtropical areas, the atmospheric transport and posterior condensation play a role on the distribution of persistent organic pollutants in the environment such as OCPs and PCBs[9], as shown by cow milk

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