



Levels of organochlorine contaminants in natural bovine milk marketed in Mojuí dos Campos and Belterra, Brazil

Isabella Clarissa Vasconcelos Rêgo¹, Julio César Amaral Cardoso¹, José Augusto Amorim Silva do Sacramento¹, Márcia Mourão Ramos Azevedo¹, Joseph Simões Ribeiro², Ruy Bessa Lopes², Lucinewton Silva de Moura², Rosivaldo de Alcântara Mendes³ and Paulo Sérgio Taube^{1*} 

¹Instituto de Biodiversidade e Florestas, Universidade Federal do Oeste do Pará, Rua Vera Paz, s/n, Salé, 68040-255, Santarém, Pará, Brazil. ²Instituto de Ciência e Tecnologia das Águas, Universidade Federal do Oeste do Pará, Santarém, Pará, Brazil. ³Laboratório de Toxicologia, Seção de Meio Ambiente, Instituto Evandro Chagas, Ananindeua, Pará, Brazil. *Author for correspondence. E-mail: pstjunior@yahoo.com.br

ABSTRACT. Analyzing the organochlorine pesticide (OCP) content in milk is relevant because it helps evaluate the quality of milk that reaches the consumer's table and also helps identify the geographical areas where there is a high possibility of contamination. Accordingly, this pilot project was aimed at determining the extent of contamination by OCP residues and their metabolites in fresh cow's milk in Mojuí dos Campos and Belterra, Pará, using solid-phase extraction (SPE) and gas chromatography coupled with electron capture detection (GC-ECD). The OCPs evaluated in this study were dichlorodiphenyltrichloroethane (DDT) and its metabolites (dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD)), hexachlorocyclohexane (HCH) (α , β , γ , and δ), endosulfan α and β , and endosulfan sulfate. None of the thirty bovine milk samples analyzed showed any contamination with OCPs. The OCP content was within the limit of quantification of the method. The results of this study add to the existing knowledge on the quality of bovine milk produced in these locations. However, further research on other environmental matrices is required to confirm the results obtained in this study.

Keywords: contamination; DDT; SPE; GC-ECD; lipids.

Received on November 30, 2021.

Accepted on March 31, 2022.

Introduction

Pesticides are used to control pests and pathogenic organisms that compromise agricultural production. However, improper application of pesticides is responsible for environmental contamination and associated public health hazards. These substances reach humans via different environmental routes and could pose health issues (Laabs, Amelung, Pinto, & Zech, 2002; Flores, Ribeiro, Neves, & Queiroz, 2004; Alves, Antoniosi Filho, Oliveira, & Furtado, 2010). Among pesticides, organochlorine compounds (OCPs) were introduced to improve agricultural production. However, OCPs persist for a long time in the environment and are highly liposoluble. This causes indirect contamination by OCP residues in food, mainly those of animal origin, via the contaminated soil, air, and water (Mello & Silveira, 2012; Polder, Skaare, Skjerve, Loken, & Eggesb, 2009; Azeredo et al., 2008; Wilson & Tisdell, 2001). Despite the prohibition on their use for agricultural purposes since the 1980s, OCPs tend to remain in places where they have been banned for many years or even where they have never been used, because they can be carried by atmospheric transport and spread by contaminated water or even food from other regions where their use is allowed (Çok et al., 2012; Azeredo et al., 2008; Zhong, Xu, Chai, & Mao, 2003).

OCPs include chlorinated diphenylmethane derivatives, such as dichlorodiphenyltrichloroethane (DDT) and its metabolites (dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), and methoxychlor, as well as hexachlorobenzene (BHC), hexachlorocyclohexanes (α -HCH, β -HCH, γ -HCH, and δ -HCH), cyclodienes (aldrin, dieldrin, endrin, chlordane, nonachlor, heptachlor, and heptachlor-epoxide), and chlorinated hydrocarbons (dodecachlor, toxaphene, and chlordecone) (D'Amato, Torres, & Malm, 2002). Foods rich in animal fat are potential sources of these persistent contaminants that bioaccumulate via the food chain (Guan et al., 2009; Gasull et al., 2010; Tsiplakou, Anagnostopoulos, Liapis, Haroutounian, & Zervas, 2010; Rêgo et al., 2019).

Milk is essential for a healthy and balanced diet and is rich in mineral salts (mainly calcium salts), lipids, carbohydrates, proteins, and vitamins (Pereira, 2014). However, milk can be contaminated by pesticides, primarily via pastures and feed exposed to such compounds, and by the improper use of veterinary products that defy good agricultural practices.

In the 1970s, the northern territory was developed by the Brazilian government, including road construction, habitation, and land-use projects, mainly along the Cuiabá-Santarém Highway (BR-163) and Belém-Brasília Highway (BR-153) (Walker, Defries, Vera-Díaz, Shimabukuro, & Venturieri, 2010). Agricultural production, especially that of soy and corn, in these regions has increased (Walker et al., 2010). However, in the municipalities of Santarém and Belterra, State of Pará, no mechanized production of soy was reported before 1990 (Barros, Lopes, Laurent, & Coelho, 2020). The introduction of crops that are not well adapted to local conditions predisposes them to compete with native vegetation, and also increases their susceptibility to fungi, insects, and other pests. This situation forces farmers to use a wide variety of pesticides, including both allowed and banned substances, such as OCPs, which are still found in clandestine markets (Waichman, 2008; Flores et al., 2004).

Torres et al. (2002) detected low concentrations (average value of $100 \mu\text{g kg}^{-1}$) of DDT in soil sourced from the Amazon region. This was attributed to its rampant use in sanitary campaigns to combat malaria. The continuous quantification of OCPs in the soil is essential for monitoring contamination sites and tracking the illegal use of these compounds (Villa, Oliveira, & Nogueira, 2011). The presence of pesticide residues and their metabolites in milk poses a risk to consumer health. Bovine milk has been used in some countries as an indicator of the presence and persistence of chemical substances in agriculture and the environment (Lemes, Kussumi, & Rocha, 2004; Luzardo et al., 2012; Avancini, Silva, Rosa, Sarcinelli, & Mesquita, 2013; Rêgo et al., 2019). The main objective of this study was to determine the contamination level of OCPs and their metabolite residues, as well as the fatty acid content in fresh cow's milk from Mojuí dos Campos and Belterra, Pará, via solid-phase extraction (SPE) and gas chromatography coupled with electron capture detection (GC-ECD).

Material and methods

All the solvents used were of HPLC grade, and other reagents, viz. acetone, acetonitrile, n-hexane, methanol, and ethyl acetate (J.T. Baker, Aparecida de Goiânia, Brazil), Milli-Q® water, Extran® MA Neutral 1% (Merck, Darmstadt, Germany), were of high purity.

Sample description

Thirty samples of fresh commercial bovine milk were collected, 15 from Mojuí dos Campos ($02^{\circ}10'17''\text{S}$ and $56^{\circ}44'42''\text{W}$) and 15 from Belterra ($02^{\circ}38'11''\text{S}$ and $54^{\circ}56'14''\text{W}$), Brazil (Figure 1), between September and November 2017. It is noteworthy that the dairy herds were relatively small (no more than 20 animals), and these farms were in the vicinity of crop production areas that mainly practiced monoculture (soy and corn).

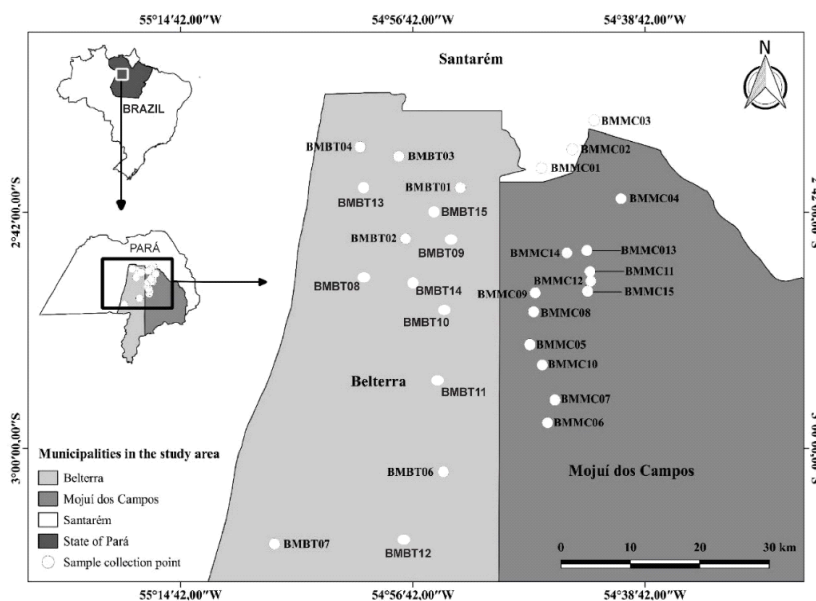


Figure 1. Beef milk collection sites in the municipalities of Mojuí dos Campos and Belterra, Pará, Brazil.

Extraction

The samples were thawed in a water bath at 37°C for 20 min., and 1 mL aliquots were placed in graduated tubes. Subsequently, 10 mL of 2:4:4 (v/v) ethyl acetate/methanol/acetone solution was added to each sample. The resulting mixture was homogenized for 1 min in a vortex (Maximix II, Thermo lyme Type 37600, São Paulo, Brazil) and then subjected to ultrasound (Branson® 5210, Chicago, USA) for 20 min. The mixture was then centrifuged (Excelsa Baby II centrifuge, Model 206-R; Nova tecnica, Piracicaba, Brazil) for 15 min. at 2000 rpm. To the supernatant, 10 mL of ultrapure water was added, and the mixture was then passed through a preconditioned (2×1 mL each of n-hexane, ethyl acetate, methanol, and ultrapure water) SPE-PACK® C₁₈ column (Sigma-Aldrich, St. Louis, USA). The mixture was passed through the column at a flow rate of approximately 6 mL min⁻¹. Finally, the column was washed twice with 1 mL of an aqueous solution of 25% acetonitrile, after which the column was vacuum-dried, and the OCPs were eluted with 2 mL of n-hexane.

The elute from the C₁₈ column was separated into two 1-mL aliquots; one aliquot was used to determine the lipid content of the sample, and the other was passed through a Florisil® column preconditioned with 10 mL each of dichloromethane, ethyl acetate, and 15% acetone/ethyl acetate solution. The pesticides were then eluted with 10 mL n-hexane and 5 mL acetone solution in 15% ethyl acetate. The total eluted volume was reduced to 1 mL using a low-pressure rotary evaporator (Rotavapor R144, Büchi Switzerland; Integrator HP 7673A). Subsequently, 100 µL of the internal standard, DCN (1,2-dichloronaphthalene), was added at a concentration of 10⁸ ng mL⁻¹.

Determination of lipid content

Since organochlorine compounds are lipophilic, they may be concentrated in the lipid fraction, making it important to determine the fat content of the milk samples. Fatty content analysis was performed during the final phase of the sample extraction. After the pesticides retained in the column were eluted with 2 mL n-hexane, 1 mL of the eluate was dried in a rotary evaporator under low pressure (Rotavapor R144, Büchi, Switzerland; Integrator HP 7673A) to determine the lipid content. All analyses were repeated in triplicates.

Conditions for chromatographic analysis

For quantitative analysis, a GC-ECD (Varian CP-3800; USA) with Workstation 5.0 (software for chromatographic data processing) was used, with a fused silica capillary column of 30 mm length, 0.32 mm ID, and 0.25 µm film thickness (OV-5, Ohio Valley Specialty Chemical, OHIO, USA). The temperature ramp from the oven to the column was programmed as follows: 150°C for 1 min. and 150 to 250°C (6°C min.⁻¹) for 5 min. The carrier gas was N₂ (99.999% pure) at a flow rate of 1.2 mL min.⁻¹. The injector was operated at 250°C in the split less mode. The detector temperature was 300°C. The qualitative analysis for the confirmation of the identified analytes was performed using a gas chromatograph coupled to a mass spectrometer (GC-MS; TSQ 8000 Thermo Scientific MS 3000; USA) with a triple-quadrupole mass analyzer operating in full-scan mode, equipped with Workstation software for processing the chromatographic data, using a fused silica capillary column (DB 05) of 30 mm length, 0.32 mm ID, and 0.25 µm film thickness. The temperature ramp from the oven to the column was programmed as 80°C for 3 min. and 80 to 280°C (10°C min.⁻¹) for 3.5 min. The carrier gas was helium (99.999% pure) set at a flow rate of 1.0 mL min.⁻¹. The injector was operated at 250°C in the splitless mode. The temperature of the quadrupole and interface was 260°C, and that of the ion source was 275°C.

Validation of analytical quality

To identify the analytes by GC-ECD, a standard solution containing all analytes was injected, and their respective retention times were determined. For quantification, an external calibration curve was constructed, and quantification was based on the integrated areas of each analytical signal (chromatographic peaks). Calibration curves were constructed to quantify each substance, with the injection of diluted solutions (2-100 ng mL⁻¹) of the analyte mixture. A Pearson's correlation index of 99% (r=0.99) was set to fit the calibration curve. Selectivity was analyzed according to the analytical signal resolution, which is related to the retention times (RTs) of the compounds. The existing resolution between various compounds was evaluated by analyzing the peak areas. The RTs were obtained from the largest peak areas present in the chromatograms of the replicates of the standard pesticide solutions. Only ten analytes were separated without coelution (Table 1). By this method, the OCPs that could be separated despite the proximity of the RTs were α-HCH (7.7 min.), β-HCH (8.5 min.), γ-HCH (Lindane) (8.7 min.), δ-HCH (9.6 min.), op'-DDE (13.7 min.), endosulfan

α (14.0 min.), pp'-DDE (14.7 min.), op'-DDD (14.9 min.), endosulfan β (15.9 min.), and pp'-DDD (16.0 min.). Endosulfan sulfate and op'-DDT coeluted at 17.0 minutes each (Figure 2). The percentage recovery was calculated by processing a sample of bovine milk with 20 ng of each OCP.

Table 1. Retention times (R) of the studied pesticides.

Pesticide	Retention Time (RT) min.
α -HCH	7.738
β -HCH	8.546
γ -HCH (Lindane)	8.680
δ -HCH	9.585
op'-DDE	13.694
Endosulfan α	14.023
pp'-DDE	14.704
op'-DDD	14.893
Endosulfan β	15.853
pp'-DDD	15.962
Endosulfan sulfate	17.006
op'-DDT	17.040

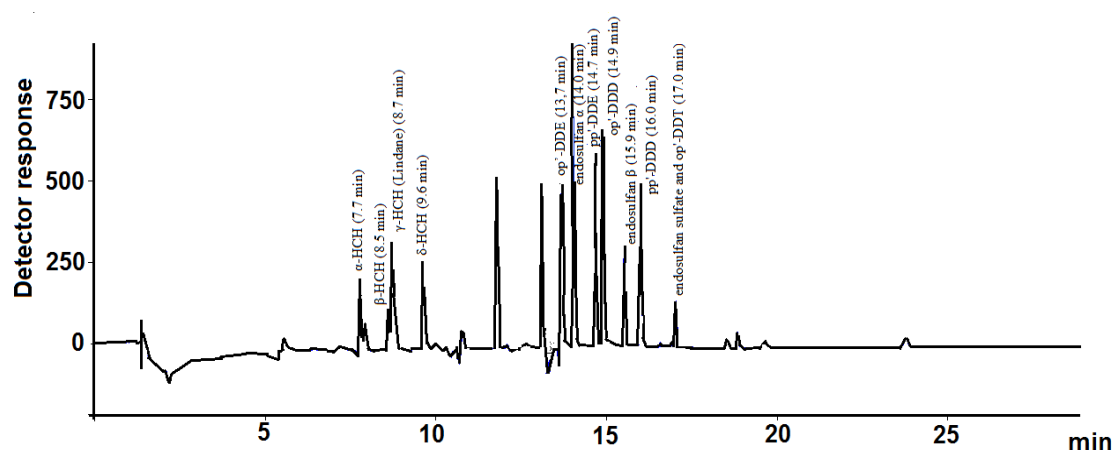


Figure 2. Chromatogram of the bovine milk sample fortified with the 12 organochlorine pesticide standards evaluated.

Results and discussion

The average fat content (% m/m) in the milk samples collected from Belterra and Mojuí dos Campos were 2.23 and 2.27%, respectively. Both samples showed significant variations in the fat content, ranging from 0.20 to 8.20% in the samples from Mojuí dos Campos and from 0.46 to 4.91% in the samples from Belterra (Table 2). The fat content of fresh bovine milk typically varies from 3.5 to 6.0% (Fangmeier, Helfenstein, & Oliveira, 2015). The differences in the lipid content within each group may be due to the varying milk composition, which is affected by lactation periods, feed, management, etc. The fat content of milk is associated with several factors, including food, climate, and the variety of cattle. Durr (2004) reported that the presence of trans-fatty acids in the diet or produced in the cow's rumen dramatically decreases the fat content of milk. This decrease occurs when there is a reduction in the ruminal pH. It is noteworthy that the water (pH 4.4–5.6) and soil (pH 4.9–5.2) in the region of Belterra and Mojuí are acidic (Aguiar, Peleja, & Sousa, 2014; Silva, Silva, Veloso, Dantas, & Sacramento, 2018; Sacramento et al., 2019).

The limits of quantification associated with this method for the evaluated OCPs varied from 0.03 to 0.05 ng g⁻¹ (Table 3). The study of linearity involves constructing calibration curves for varying standard concentrations as per the concentration range determined for each pesticide. The calibration curve allows parameters, such as the correlation coefficient (*r*), to be obtained (Paschoal et al., 2008; Barbosa, 2012). Accordingly, linearity was assessed using the internal calibration curve method in the range from 2 to 100 ng mL⁻¹ of the standard solution containing all the analytes under study. The Pearson correlation index for accepting the calibration curve was 99% (*r* = 0.99), implying that the internal calibration curve showed analyte signals proportional to the signal areas. The accuracy of the chromatographic method was evaluated by

analyzing the analyte recovery and matrix effects. This method is used to assesses the extraction yield, i.e. the mass fraction of the analyte in the sample present in the final extract. For the analysis of organic compounds, the accuracy was measured using recovery tests.

Table 2. Lipids content in the evaluated samples (% m m⁻¹).

Sample	Lipids content (% m m ⁻¹)	Sample	Lipids content (% m m ⁻¹)
CMB 01	2.32 ± 0.12	CMM 01	1.26 ± 0.12
CMB 02	2.79 ± 0.15	CMM 02	1.51 ± 0.05
CMB 03	1.86 ± 0.22	CMM 03	1.57 ± 0.04
CMB 04	1.52 ± 0.12	CMM 04	1.52 ± 0.11
CMB 05	4.91 ± 0.09	CMM 05	3.53 ± 0.14
CMB 06	1.31 ± 0.17	CMM 06	1.64 ± 0.12
CMB 07	3.02 ± 0.15	CMM 07	2.57 ± 0.14
CMB 08	2.55 ± 0.13	CMM 08	0.99 ± 0.25
CMB 09	3.95 ± 0.10	CMM 09	0.76 ± 0.15
CMB 10	0.61 ± 0.27	CMM 10	1.14 ± 0.17
CMB 11	1.86 ± 0.09	CMM 11	0.20 ± 0.15
CMB 12	1.64 ± 0.19	CMM 12	1.67 ± 0.12
CMB 13	0.46 ± 0.24	CMM 13	8.20 ± 0.56
CMB 14	2.23 ± 0.16	CMM 14	4.47 ± 0.13
CMB 15	2.36 ± 0.09	CMM 15	3.07 ± 0.10
Average	2.23	Average	2.27
Standard deviation	1.16	Standard deviation	1.99

CMB: cow milk from Belterra. CMM: cow milk from Mojuí dos Campos.

Table 3. Detection and quantification limits of the evaluated organochlorine pesticides (ng g⁻¹) in the municipalities of Mojuí dos Campos and Belterra, Pará, Brazil.

ID sample	pp'-DDT	op'-DDT	pp'-DDD	op'-DDD	pp'-DDE	op'-DDE	α-HCH	β-HCH	δ-HCH	γ-HCH	Endosulfan α	Endosulfan α	Endosulfan sulfate
LOD	0.002	0.002	0.002	0.002	0.001	0.001	0.010	0.010	0.010	0.010	0.020	0.020	0.020
LOQ	0.010	0.010	0.010	0.010	0.005	0.005	0.040	0.040	0.040	0.040	0.050	0.050	0.050

LOD: limit of detection. LOQ: limit of quantification.

The recovery of analytes is defined by the analysis of samples fortified with known quantities, but the parameter generally depends on the concentration; therefore, it must be evaluated within the range of concentrations expected for the sample (Kurz, 2007; Leite, 2011). The recoveries obtained for all the OCPs ranged from 48 to 91% (Table 4). Complex matrix recoveries of around 70–130% are expected, implying that some samples showed lower than desirable recovery values, possibly due to losses during the sample preparation. Unfortunately, the recovery of six non-polar analytes could not be evaluated, perhaps due to the polarity of the solvent (methanol) used during the elution stage of the SPE. Owing to the low polarity of these compounds, the solvent may not have enough strength (polarity) to elute them from the C₁₈octadecyl silica SPE Pack® column cartridge, thus retaining them in the cartridge (Halimah et al., 2008).

Table 4. Recovery (%) of the pesticides studied in the municipalities of Mojuí dos Campos and Belterra, Pará, Brazil.

Pesticide	Recovery (%)
α-HCH	65 ± 3.5
β-HCH	58 ± 3.2
γ-HCH (Lindane)	71 ± 3.6
δ-HCH	48 ± 3.0
op'-DDE	73 ± 3.5
Endosulfan α	84 ± 3.4
pp'-DDE	86 ± 3.6
op'-DDD	63 ± 3.3
Endosulfan β	89 ± 3.8
pp'-DDD	91 ± 4.1
Endosulfan sulfate	70 ± 3.7
pp'-DDT	80 ± 3.5

None of the samples analyzed showed any contamination by the OCPs within the limits of detection and quantification of the method. This study contributes to the knowledge regarding the quality of fresh bovine milk from the municipalities of Mojuí dos Campos and Belterra. The results of this work were compared with

other similar studies carried out in different places across the world where DDT and other OCPs were widely used. Most samples from such locations showed contamination by OCPs (Table 5).

Table 5. Works related to the evaluation of the content of organochlorine pesticides in bovine milk in different regions of the world.

Authors	Site	Year	Sample	Organochlorinepesticidesdetected
Lemes et al.	São Paulo, Brazil	2004	Conventional bovine milk	None
Avancini et al.	Mato Grosso do Sul, Brazil	2013	Conventional bovine milk	Aldrin, DDT, mirex, endosulfan, chlordane, dicofol, heptachlor and dieldrin
Heck, Santos, Bogusz Junior, Costabeber, and Emanuelli	Rio Grande do Sul, Brazil	2007	Fresh and pasteurized bovine milk (UHT)	α -HCH, lindane, aldrin, HCB, p,p'-DDE, o,p'-DDD, p,p'-DDD e o,p'-DDT and PCB (congeners 10, 28, 52, 138 e 180).
Luzardo et al.	Ilhas Canárias, Espanha	2012	Conventional and organic bovine milk	p,p'-DDE, aldrin, cis and trans-chlordane, dieldrin, endrin, Heptachlor, HCB and endosulfan α e β . HCH, DDT, and aldrin
Zhong et al.	China	2003	Commercial bovine milk	DDT, DDE e pp'-DDD, HCH (α , β e γ), heptachlor epoxide and aldrin
John, Bakore, and Bhatnagar	Ragastão (Índia)	2001	Commercial bovine milk	DDT, DDE e pp'-DDD, HCH (α , β e γ), heptachlor epoxide and aldrin
Hernandez et al.	São Pedro (Colômbia)	2010	Fresh bovine milk	DDT, endrin, endosulfan, γ -chlordane, heptachlor epoxide.
Gutiérrez et al.	Chiapas (México)	2012	Organic bovine milk	HCH (α , β e γ), heptachlor epoxide, DDT and aldrin.
Darko, Akoto, and Oppong	Kumasi (Gana)	2008	Fresh bovine milk	Aldrin, dieldrin, endosulfan, and DDT
Kampire, Kiremire, Nianzi, and Kishimba	Kampala	2011	Fresh and pasteurized bovine milk (UHT)	Aldrin, dieldrin, endosulfan, Lindane, and DDT

The results of this study are similar to that from the São Paulo State (Lemes et al., 2004); however, it differs from those conducted at Rio Grande do Sul and Mato Grosso do Sul by Heck et al. (2007) and Avancini et al. (2013), respectively. Further, the results of this work is also unlike those reported by John et al. (2000) in India, Zhong (2003) in China, Darko et al. (2008) in Ghana, Hernández, Vidal, and Marrugo (2010) in Colombia, Kampire et al. (2011) in Kampala, Gutiérrez, et al. (2012) in Mexico and Luzardo et al. (2012) in Spain. Lemes et al. (2004) did not detect any OCPs or any of their metabolites in 73 samples of commercial bovine milk in the state of São Paulo. Avancini et al. (2013) evaluated organochlorine compounds in bovine milk in the state of Mato Grosso do Sul, and of the total samples analyzed, more than 90% were contaminated with at least one OCP residue. In Rio Grande do Sul, Heck et al. (2007) analyzed samples of fresh pasteurized and ultra-pasteurized (or ultra-high-temperature pasteurized) milk. In all the analyzed samples, α -HCH, lindane, aldrin, HCB, pp'-DDE, op'-DDD, pp'-DDD, and op'-DDT, and PCB (congeners 10, 28, 52, 138, and 180) were found.

Conclusion

None of the bovine milk samples analyzed in this study showed any contamination by OCPs. This may be due to the absence of pesticide residues in the feed consumed by the cattle. Although the results obtained indicate no OCP contamination in bovine milk, more studies are required for a more comprehensive understanding of the OCP contamination in the studied region. It is important to highlight the relevance of milk-monitoring studies in assessing the presence of pesticide residues in foodstuff.

Acknowledgments

This work was supported by Project 88881.159143/2017-01 funded by the Coordination of Improvement of Higher Education Personnel (CAPES) and the Amazonia Foundation for Studies and Research in Pará (FAPESPA). The authors also thank two anonymous reviewers for their constructive comments and suggestions. We also would like to thank Editage (www.editage.com) for English language editing.

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**Levels of organochlorine contaminants in natural bovine
milk marketed in Mojuí dos Campos and Belterra, Brazil**

Acta Scientiarum. Animal Sciences
vol. 45, e61721, 2023
Editora da Universidade Estadual de Maringá - EDUEM,
ISSN: 1806-2636
ISSN-E: 1807-8672

DOI: <https://doi.org/10.4025/actascianimsci.v45i1.61721>