CIÊNCIA e NATURA
ISSN 2179-460X
OPEN ACCESS
Ci. e Nat., Santa Maria, v. 46, e73976, 2024 • https://doi.org/10.5902/2179460X73976
Submitted: 01/31/2023 • Approved: 09/12/2024 • Published: 11/29/2024

**Environment**

# Monitoring carbamate and organophosphate pesticides in pasteurized milk

## Monitoramento de resíduos de carbamatos e organofosforados em leite pasteurizado

**Maria Antônia Torres de Bari** [I], **Ana Paula Kuller Zanoni** [II], **Ronaldo Tamanini** [I] **Tatiane Cargnin Faccin** [I], **Natália Gonzaga** [I], **João Victor Sobreira Xavier** [I], **Glaura Scantamburlo Alves Fernandes** [I], **Rafael Fagnani** [I]

[I] Universidade Estadual de Londrina, Londrina, PR, Brazil
[II] Universidade Norte do Paraná, Londrina, PR, Brazil

## ABSTRACT

Milk is an essential and widely consumed food. In 2022, Brazil produced approximately 25 billion liters of milk for human consumption, and contamination by agrochemicals can have significant impacts on public health. This study analyzed organophosphate and carbamate pesticide residues, as well as their physicochemical and microbiological quality, in pasteurized milk sold in Londrina and Arapongas, Brazil. From June to December 2017, 35 samples of pasteurized milk were obtained from the commercial market and analyzed by HPLC and physical-chemical and microbiological tests for freezing point, alkaline phosphatase, lactoperoxidase, and Enterobacteriaceae count. All the samples met the maximum residue limits for both classes of agrochemicals, indicating safe consumption and good agricultural practices. However, the potential effects of degradation byproducts on human health from milk consumption require further investigation. Regarding the physicochemical and microbiological quality, 9% of the samples showed post-pasteurization contamination and 3% indicated water addition. Thus, it is concluded that the biggest challenge for pasteurized milk production industries is to avoid post-pasteurization microbiological contamination, and better tools and auditing are necessary to prevent and detect residual water.

**Keywords**: Dairy; Chemical residues; Post-pasteurization contamination

## RESUMO

O leite é um alimento essencial e amplamente consumido. Em 2022, o Brasil produziu aproximadamente 25 bilhões de litros de leite para consumo, sendo que contaminações por agroquímicos podem ter consequências impactantes à saúde pública. Este estudo analisou resíduos de pesticidas organofosforados e carbamatos, bem como analisou sua qualidade físico-química e microbiológica em leite pasteurizado comercializado em Londrina e Arapongas, Brasil. Entre junho e dezembro de 2017, 35 amostras de leite pasteurizado foram obtidas do comércio e analisadas por HPLC , além de testes físico-químicos e microbiológicos para ponto de congelamento, fosfatase alcalina, lactoperoxidase e contagem de Enterobacteriaceae. Todas as amostras atenderam aos limites máximos de resíduos para ambas as classes de agroquímicos, indicando consumo seguro e boas práticas agrícolas. No entanto, os efeitos potenciais dos subprodutos da degradação na saúde humana a partir do consumo de leite requerem uma investigação mais aprofundada. Quanto à qualidade físico-química e microbiológica, 9% das amostras apresentaram contaminação pós pasteurização e 3% indicaram adição de água. Dessa forma, conclui-se que o maior desafio para as indústrias produtoras de leite pasteurizado seja evitar a contaminação microbiológica pós pasteurização, sendo necessário melhores ferramentas e auditoria para prevenir e detectar resíduos de água.

**Palavras-chave**: Laticínio; Agroquímicos; Contaminação pós-pasteurização

## 1 INTRODUCTION

Milk is an important and widely consumed food. In 2022, Brazil produced about 25 billion liters of milk for human consumption (Instituto Brasileiro de Geografia e Estatística [IBGE], 2022). The manufacture of pasteurized milk is very common among Brazilian dairy plants, present in about 1 in 4 dairy establishments in the Brazilian Federal Inspection Service (Lima, Perez, & Chaves, 2017). To accomplish the high demand of milk consumption, the milk farming can demand many pesticides, which can pass readily from animal feed to milk. Most of the time, the animal feed is the main source of milk contamination, but other factors may include environmental exposure, application of ectoparasites and accidental spills (Li, Xiong, & Fantke, 2022).

The presence of pesticide residues can negatively affect human health. Both carbamates and organophosphorus act by inhibiting the acetylcholinesterase in the nervous system, impairing the respiratory, myocardial, and neuromuscular transmission. Their bioaccumulation can further cause neurodegenerative and allergic diseases, metabolic disturbances, endocrine disruptions, obesity, type 2 diabetes, renal diseases, neonatal developmental abnormalities, DNA damages and cancers (Sarlak et al., 2021).

A recent systematic review about pesticide in milk across the globe since 2010 pointed to the existence of considerable risks, mainly regarding organochlorine, but without discard organophosphate and carbamate pesticides (Boudebbouz et al., 2022). Thus, continuous monitoring is necessary for the assessment of adverse effects of pesticide residues on human health.

In Brazil, few studies have investigated pesticide residues in commercial milk. At national level, the program on Pesticide Residue Analysis in Food (PARA), coordinated by the National Sanitary Surveillance Agency (Anvisa), covers only plant foods (Agência Nacional de Vigilância Sanitária [ANVISA], 2017). Another national monitoring program is the National Residue and Contaminant Control Program (PNCRC), coordinated by Ministry of Agriculture, Livestock and Food Supplies (MAPA), which englobe raw milk, but does not cover commercial milks, such as pasteurized and UHT (Plano Nacional de Controle de Resíduos e Contaminantes [PNCRC], 2021). Thus, there is a gap regarding analysis of pesticides residues in milk for direct consumption.

Considering the relevant role of pasteurized milk in the world dairy supply, our study aimed to investigate the chemical and the microbiological contamination of pasteurized milk sold in Londrina and Arapongas, Brazil.

## 2 MATERIAL AND METHODS

### 2.1 Sampling

All supermarkets' chains of Londrina and Arapongas were considered, retrieving its location from the on-line phone book. Sixteen catalogued supermarkets were vised by a trained team to visually inspect the displays stands of the whole store, looking for Brazilian brands of pasteurized milk.

The sampling included all brands (5) of full fat (3%) pasteurized milk sold in Londrina and Arapongas between June and December 2017, manufactured and packed at dairy plants from Paraná State (3) and São Paulo State (2) under state or federal inspection.

The on-site visit methodology was performed fivefold to include different batches of the same brand, accounting for randomized effects.

A total of 35 commercial samples of pasteurized milk (7 batches of 5 Brazilian brands) were purchased, cooled (<10ºC) with ice packs and immediately transported to the laboratory. In addition, 10 milk samples were from the market surveillance conducted by the Brazilian Health Regulatory Agency.

## 2.2 Extraction and HPLC analysis

The pasteurized milk samples (10mL) were mixed with 10 mL of formic acid solution (0.025% v/v) and vortexed for 1 min. To this solution, 10 ml of acetonitrile was added, vortexed for 1 min and centrifugated at 3,000 x g for 15 minutes. The supernatant was carefully separated into another test tube, and 2 mg of magnesium sulphate was added to the extract and centrifugated at 3,000 x g for 15 minutes. The supernatant was microfiltered by nylon microfiltration membrane (0.22 µm) and then analyzed by liquid chromatography.

The HPLC analysis was carried out on a Waters Alliance 2695 HPLC system coupled with a Waters 2998 detector. The separation of pesticide residues was carried out on the ACE C18 column (ACE, Scotland) of length 150 mm, 4.6 mm internal diameter and 5µm particle thickness. The mobile phase consisted of acetonitrile and water (1:1) was filtered, degassed and pumped at a flow rate of 0.5 $mL.min^{-1}$. The column oven was set at 35 °C and the injected volume was 20 µL, UV detector set at 285 nm. Retention time for CFF was 4.88 min, and analytical run was 7.0 min.

The standard used for organophosphorus were: Chlorpyrifos, Diazinon, Dichlorvos, Dimethoate, Fenthion, and Monocrotophos. Aldicarb, Bendiocarb, Carbaryl, Carbofuran, Carbosulfan, Methomyl, Propoxur and Thiodicarb were used for carbamates, all purchased from Sigma Aldrich (St Louis, MO, USA).

Blank milk samples (milked by our team) were spiked with three levels of each pesticide. The recoveries rates were more than 70% (ranging from 70 to

103%). A signal-to-noise ratio of 2:1 was considered for estimating the detection limit. All pesticides showed a limit of detection (LOD) of 1 μg/L.

The limit of quantification (LOQ) was based on the signal-to-noise approach, comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing 10:1 as the minimum concentration. The LOQ for Chlorpyrifos, Diazinon, Dichlorvos, Dimethoate, Fenthion, Aldicarb, Bendiocarb, Carbaryl, Carbofuran, Carbosulfan, Methomyl was 5 μg/L. The LOQ for Monocrotophos, Propoxur and Thiodicarb was 10 μg/L.

### 2.3 Compositional and microbiological analysis

The samples were analyzed for: (1) freezing point assessment by thermistor cryoscope (PZL 7000S, Londrina, Brazil) (ISO 5764:2009); (2) alkaline phosphatase (AOAC, 2016) and (3) lactoperoxidase (ISO, 2011). The results from compositional analysis were based on the percentage of samples that were compliant to the parameters set in the statutory Brazilian regulation for pasteurized milk.

The samples were serially diluted with sterile buffered peptone water and plated in duplicated to be used in colony-counting of enterobacteria using 3M Petrifilm Enterobacteriaceae Count (EB) plates. Incubations were performed at 37 °C for 24 h.

## 3 RESULTS AND DISCUSSION

All 35 samples from seven batches are found to comply the chemical specifications for organophosphate and carbamate pesticides (100% < LOQ). Just two samples (6%) were positive in traces (LOD < x < LOQ) for Dimetoate.

The occurrence of pesticides is more common in raw milk rather than pasteurized or UHT milk (Calahorrano-Moreno et al., 2022). It is well known that some pesticides are affected by heat treatment and/or fermentation (Bo et al. 2011). In addition, the degradation kinetics of organophosphorus may also be influenced by some lactic acid bacteria naturally present in milk, such as *Lactobacillus* spp. (Yuan et al., 2021).

Photodegradation by sun light is another mechanism that could occur through the dairy chain, and consequently, support the low levels of pesticides found here (Ishag et al., 2019).

Few studies have investigated pesticide residues in commercial milk from Brazil, most of them monitoring organochlorine pesticides levels (Régo et al., 2019). Our study disclosed a decrease trend compared to the most recent monitoring study that evaluated 79 pesticides in organic and conventional milk, where Granella et al. (2013) found about 9% of samples contaminated with clomazone, chlorpyrifos or monocrotophos.

From our results, there is no health risk of human exposure to pesticides through pasteurized milk consumption. However, some pesticides degradation, might lead to the metabolites more active than the parent compounds, which were not evaluated in this study and deserves further attention (Tang, Rose, & Chambers, 2006).

About 9% of samples were above the statutory limit of 10 CFU/mL for *Enterobacteria* spp. The mean value and standard deviation for *Enterobacteria* spp. count was 1.67 (±3.69) MPN/mL, ranging from <1 to 14 CFU/mL.

The compliance of the microbiological criterium was a challenge for the most of dairy plants included in our study, once four of five brands showed at least one sample with microbiological contamination. All samples in disagreement with the requirements of *Enterobacteria* spp. were negative for alkaline phosphatase, indicating post-pasteurization contamination. Most of Enterobacteriaceae are environmental contaminants, but a fraction of this group is from fecal contamination, which represents poor hygienic conditions through milk processing, or from enteropathogens, such as *Escherichia coli* and *Salmonella*, types of microbiological contaminants that threaten food safety (Martin et al., 2016).

Thus, it is possible to assume that the post-pasteurization contamination is a considerable barrier for some dairy processors included in this study. The three main reasons for this kind of issue are flaws in: (1) cleaning and sanitization protocols; (2) control of cross contamination, and (3) preventive maintenance. Thus, efforts are clearly needed to reduce all post-processing contaminations. The root cause-analysis and the source tracking of contamination are good approaches to identify the causative elements of post-pasteurization contamination. In addition, molecular tools, such as pulsed field electrophoresis, ribotyping, multilocus sequence typing, and DNA-based techniques are useful to discriminate between persistent (i.e., biofilms) and transient contaminations (i.e., cross contamination) (Martin, Boor, & Wiedmann, 2018; Zoellner, Ceres, Ghezzi-Kopel, Wiedmann, & Ivanek, 2018; Nakamura et al., 2021).

Regarding the freezing point, about 3% of the samples were above the statutory limit of -0.530ºH, an indication of added water. This non-conformity can be from deliberated addition of water, an economical food fraud, or from stagnant residual water caused by rinse failures or problems with the clean in place system design of the dairy plants (Ansari, Chavan & Bhatt, 2018). Thus, the Brazilian inspection services should drive efforts not only to combat the deliberated water addition, but also routinely audit the current CIP elements at dairy plants, crucial to ensure the operational efficiency and the pasteurized milk quality.

## 4 CONCLUSIONS

The low levels of carbamate and organophosphate residues in pasteurized milk showed no risk for consumers. The data indicate good agriculture practices or pesticide degradation throughout the dairy chain. The potential effects of active metabolites from pesticides degradation on human health from pasteurized milk consumption are not clear and require further investigation.

There is a challenge for most of dairy plants to avoid the microbiological contamination, which highlights the need for better tools to minimize the post-pasteurization contamination, as well as enhance the audit to improve residual water prevention and detection.

## ACKNOWLEDGEMENTS

The authors thank the Instituto Nacional de Ciência e Tecnologia para a Cadeia Produtiva do Leite.

## REFERENCES

Ansari, M. I. A., Chavan, R. S., & Bhatt, S. B. (2018). Fouling of milk and cleaning-in-place in the dairy industry. In *Technological Interventions in Dairy Science*, 179-206. Apple Academic Press.

Agência Nacional de Vigilância Sanitária (ANVISA). (2019). *Agrotóxicos e toxicologia: Programa de Análise de Resíduos de Agrotóxicos em Alimentos – PARA: Plano Plurianual 2017-2020 – Ciclo 2017/2018*, 136. Brasília. Retrieved from: http://portal.anvisa.gov.br/programa-de-analise-de-registro-de-agrotoxicos-para

Association of Official Analytical Chemists (AOAC). (2016). *Official methods of analysis of AOAC international* (20th ed.). Official Method AOAC 965.26. Arlington: AOAC International.

Bo, L. Y., Zhang, Y. H., & Zhao, X. H. (2011). Degradation kinetics of seven organophosphorus pesticides in milk during yoghurt processing. *Journal of the Serbian Chemical Society, 76(3)*, 353-362.

Boudebouz, A., Boudalia, S., Boussadia, M. I., Gueroui, Y., Habila, S., Bousbia, A., & Symeon, G. K. (2022). Pesticide residues levels in raw cow's milk and health risk assessment across the globe: A systematic review. *Environmental Advances,* 9, 100266.

Calahorrano-Moreno, M. B., Ordoñez-Bailon, J. J., Baquerizo-Crespo, R. J., Dueñas-Rivadeneira, A. A., Montenegro, M. C. B., & Rodríguez-Díaz, J. M. (2022). Contaminants in the cow's milk we consume? Pasteurization and other technologies in the elimination of contaminants. *F1000 Research, 11*, 1-34.

Granella, V., Ventorini, C. G., Pigatto, G. M., & Nörnberg, J. L. (2013). Pesticide residues in organic and conventional pasteurized milks. *Semina: Ciências Agrárias, 34*(4), 1731-1740.

Instituto Brasileiro de Geografia e Estatística (IBGE). (2020). Censo *Agropecuário*. Brasília: Ministério do Planejamento, Orçamento e Gestão. Retrieved from: http://www.ibge.gov.br/home/estatistica/economia/agropecuaria/indicadoresagro_19962003/default.shtm

Ishag, A. E. S. A., Abdelbagi, A. O., Hammad, A. M. A., Elsheikh, E. A. E., & Hur, J. H. (2019). Photodegradation of chlorpyrifos, malathion, and dimethoate by sunlight in the Sudan. *Environment Earth Science, 78*, 1-14.

International Organization for Standardization (ISO). (2009). *ISO 5764:2009. Milk - Determination of freezing point - Thermistor cryoscope method.* Geneva, Switzerland: ISO.

International Organization for Standardization (ISO). (2011). *ISO 17193:2011. Milk - Determination of the lactoperoxidase activity – Photometric method.* Geneva, Switzerland: ISO.

Li, Z., Xiong, J., & Fantke, P. (2022). Screening of pesticide distributions in foods of animal origin: A matrix-based approach for biotransfer factor modeling of grazing mammals. *Environmental Science: Processes & Impacts, 24*(4), 609-624.

Lima, L. P. de, Perez, R., & Chaves, J. B. P. (2017). The dairy industry in Brazil-an exploratory study. *Boletim do Centro de Pesquisa e Processamento de Alimentos, 35*(1).

Martin, N. H., Trmčić, A., Hsieh, T. H., Boor, K. J., & Wiedmann, M. (2016). The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. *Frontiers in Microbiology,* 7.

Martin, N. H., Boor, K. J., & Wiedmann, M. (2018). Symposium review: Effect of post-pasteurization contamination on fluid milk quality. *Journal of Dairy Science, 101*(1), 861-870.

Nakamura, A., Takahashi, H., Arai, M., Tsuchiya, T., Wada, S., Fujimoto, Y., & Kimura, B. (2021). Molecular subtyping for source tracking of Escherichia coli using core genome multilocus sequence typing at a food manufacturing plant. *PLOS ONE, 16*(12), e0261352.

Ministério da Agricultura e Pecuária (MAPA). (2021). *Plano Nacional de Controle de Resíduos e Contaminantes.* Brasília. Retrieved from https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-animal/plano-de-nacional-de-controle-de-residuos-e-contaminantes/consolidado-resultados-pncrc-2021.pdf

Régo, I. C. V., Santos, G. N. V. D., Ribeiro, J. S., Lopes, R. B., Santos, S. B. D., & Vasconcelos, A. A. (2019). Organochlorine pesticides residues in commercial milk: A systematic review. *Acta Agronómica, 68*(2), 99-107.

Sarlak, Z., Khosravi-Darani, K., Rouhi, M., Garavand, F., Mohammadi, R., & Sobhiyeh, M. R. (2021). Bioremediation of organophosphorus pesticides in contaminated foodstuffs using probiotics. *Food Control, 126*, 108006.

Tang, J. U. N., Rose, R. L., & Chambers, J. E. (2006). Metabolism of organophosphorus and carbamate pesticides. In *Toxicology of Organophosphate & Carbamate Compounds*, 127-143. Academic Press.

Yuan, S., Yang, F., Yu, H., Xie, Y., Guo, Y., & Yao, W. (2021). Biodegradation of the organophosphate dimethoate by *Lactobacillus plantarum during milk fermentation. Food Chemistry, 360*, 130042.

Zoellner, C., Ceres, K., Ghezzi-Kopel, K., Wiedmann, M., & Ivanek, R. (2018). Design elements of Listeria environmental monitoring programs in food processing facilities: A scoping review of research and guidance materials. *Comprehensive Reviews in Food Science and Food Safety, 17*(5), 1156-1171

## Authorship contributions

### 1 – Maria Antônia Torres de Bari

Graduated in Veterinary Medicine from the State University of Londrina (UEL).
https://orcid.org/0000-0001-6247-9203 • maria.antonia.torres@uel.br
Contribution: Data curation, Formal Analysis, Investigation.

### 2 – Ana Paula Kuller Zanoni

Master's student in Animal Health and Production at Universidade Pitágoras-Unopar; Graduated in Veterinary Medicine at Centro Universitário Filadélfia - UniFil.
https://orcid.org/0000-0002-2595-3858 • anapaulakzanoni@gmail.com
Contribution: Conceptualization, Resources, Project administration, Supervision, Validation, Visualization, Writing - review & editing.

### 3 – Ronaldo Tamanini

Master's degree in animal science from UEL (2008); PhD in Animal science.
https://orcid.org/0000-0002-4497-9164 • ronaldot@uel.br
Contribution: Methodology, Writing – original draft.

### 4 – Tatiane Cargnin Faccin

PhD in Veterinary Medicine (2017) at the Federal University of Santa Maria.
https://orcid.org/0000-0003-4989-9515 • tatiane.faccin@uel.br
Contribution: Methodology, Writing – original draft.

### 5 – Natália Gonzaga

PhD degree in Animal Science from the State University of Londrina.
https://orcid.org/0000-0001-9310-7496 • nataliagonzaga@uel.br
Contribution: Writing – original draft, Writing – review editing.

### 6 – João Victor Sobreira Xavier

Veterinarian graduated from Centro Universitário Filadélfia - UniFil.
https://orcid.org/0000-0002-0515-3247 • joaovictor.xavier@uel.br
Contribution: Methodology, Writing – original draft.

### 7 – Glaura Scantamburlo Alves Fernandes

Doctorate (2009) in Cellular and Structural Biology from UNICAMP.
https://orcid.org/0000-0002-6043-1131 • glaura@uel.br
Contribution: Conceptualization, Project Administration, Resources, Supervision, Validation, Visualization, Writing–review editing.

**8 – Rafael Fagnani**

PhD (2013) in Animal Science; Professor at Universidade Anhanguera - Unopar in State University of Londrina.
https://orcid.org/0000-0002-7392-2087 • rafaelfagnani@hotmail.com
Contribution: Conceptualization, Resources, Project administration, Supervision, Validation, Visualization, Writing - review & editing.

## How to quote this article

Bari, M. A. T. de., Zanoni, A. P. K., Tamanini, R., Faccin, T. C., Gonzaga, N., Xavier, J. V. S., Fernandes, G. S. A., & Fagnani, R. (2024). Monitoring carbamate and organophosphate pesticides in pasteurized milk. *Ciência e Natura*, Santa Maria, 46, e73976. DOI:10.5902/2179460X73976. Disponível em: https://doi.org/10.5902/2179460X73976