

## Ochratoxin A in Infant Food from Amazon Region in Brazil

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### Abstract

Children's foods have diversified both in terms of flavor options and practicality to attract consumers. However, sanitary aspects involving the presence of toxic agents such as mycotoxins, substances with carcinogenic effects, must be considered. As the child consumer is still in body formation and whose exposure limit to contaminants needs to be assessed, the objective of the work was to monitor the occurrence of contamination by ochratoxin A in children's foods produced in the Amazon region - Brazil. The samples were analyzed by liquid chromatography and the results showed that 16.7% of the samples were contaminated. As this was pioneering work, we suggest that monitoring be a routine adopted by government agencies and that producing companies seek to qualify their suppliers and processes to guarantee safe food for children.

**Keywords:** mycotoxin, cereal, health

### 1. Introduction

It is wellknown infant feeding practices impact children's nutritional and health status, influencing growth and development (Andrade et al., 2021). In many countries, cereals such as barley, oats, rice, and wheat are introduced as the first solid foods for babies to support nutrition and partially replace breast milk or infant formula in the diet. Some of them are frozen foods obtained using other technologies that attract consumers because they are practical, taking less time to prepare. Even in this modern life scenario, the composition and toxicology aspects of infant food require attention. When monitoring the occurrence of contaminants in baby foods, it is important to consider toxicology, including the presence of mycotoxins such as Ochratoxin A (OTA), a carcinogen produced by some fungi (Kumar et al., 2020). OTA can occur in foods formulated with different types of grains (barley, rice, oats, wheat, and mixed grains), resulting from contamination by ingredients, processing, or storage of the food (Pereira et al., 2022; Vin et al., 2020). Mycotoxins originated from raw materials such as cereals and fruits used in baby foods are difficult to remove or eliminate once formed, in this sense, the best control is prevention and mitigation strategies (Hernandes et al., 2021). Traditionally, in European countries, wheat products are the first solid foods consumed by babies from the early stages of weaning. Therefore, child consumers have higher cereal intakes than adults in relation to body weight. The quality of processed baby foods necessarily depends on control of all stages of production. Children older than 5 months may be more vulnerable to the toxic effects of mycotoxins than adults, due to their lower body weight, higher metabolic rate, reduced ability to detoxify food toxins and more restricted diet. Due to the possible risk associated with the consumption of OTA by infants, several countries have established regulatory limits for OTA in cereal-based foods for infant feeding (infants and young children), including Brazil, which established in 2011 for immediate application, the maximum limit of 2µg/kg OTA (Brazil, 2011; Capozzo et al., 2017; Bonerba et al., 2017). In Brazil, the monitoring of mycotoxins in baby foods has prioritized the investigation of breast milk with several works (Coppa et al., 2021; Frey et al., 2021). However, assessment of mycotoxins in baby foods is still a scarce subject in Brazil, especially addressing specific geographic areas such as the Amazon. Faced with this challenging scenario in investigating the contamination of children's foods, the objective of the work was to evaluate the contamination of OTA in children's foods produced in the Brazilian Amazon region and obtain data that can assist in public health prevention strategies.

## 2. Method

### 2.1 Sampling

Infant foods ( $n = 30$ ) frozen, from different brands, and sold in local stores (retail), were purchased in the city of Manaus- Am -Brazil, in 2022-2023, and those produced from cereals and composition of other ingredients from the North region were selected, such as fish and bovine meat. The samples were kept refrigerated or frozen at  $-15^{\circ}\text{C}$  in a freezer as indicated on the product labeling, until the moment of each duplicate analysis. The samples were recorded according to flavor, ingredients, date of manufacture and were valid for 6 months and were analyzed at the Center for Studies in Composition and Toxicology and Food at the Faculty of Pharmaceutical Sciences-UFAM. The original packaging was made of polypropylene with a net weight of between 200 and 300g. The most frequent ingredients are described in table 1.

Table 1. Ingredients declared in the samples labeling

Ingredient	Declared in the samples labeling (%)
Fish	20
Fruits	30
Meat (chicken or bovine)	50
Cereals	100
Rice	80

### 2.2 Ochratoxin A Analysis

(a) Preparation of ochratoxin A working solution: the Supelco <sup>®</sup> OTA standard (50 ng / $\mu\text{l}$ ) was diluted in 20  $\mu\text{l}$  of OTA (50 ng / $\mu\text{l}$ ) + 980  $\mu\text{l}$  of ethyl alcohol = 1 ng / $\mu\text{l}$ . The OTA 1 working solution (1 ng / $\mu\text{l}$ ) was then diluted, 100  $\mu\text{l}$  of OTA 1 working solution (1 ng / $\mu\text{l}$ ) + 900  $\mu\text{l}$  of ethyl alcohol = 0.1 ng / $\mu\text{l}$ . The calibration curve was obtained by diluting the ochratoxin 2 working solution with methanol to obtain concentrations (ng/g).

2.3 *Sample extraction and cleaning*: the method described by the manufacturer Romer was used for extraction and cleaning. Labs. An aliquot of 25 g of blank/sample was extracted with 100 ml of acetonitrile /water (84:16 v/v) for 3 minutes. An aliquot was filtered, and 7 mL acidified with 70 $\mu\text{L}$  of acetic acid. The acidified extract was loaded onto a Mycosep 229 <sup>®</sup> column Ochratoxin and then 1.5 ml of water was added before injecting into the chromatograph.

### 2.4 Quantification in High-performance Liquid Chromatography

The Agilent 1100 Series instrument was used, equipped with pumps, with a Rheodyne Model 7125 injector (100  $\mu\text{l}$  loop) and a fluorescence detector. A Restek C18 (5  $\mu\text{m}$ ) LC column (250 mm x 4.6 mm i.d.) used with a mobile phase consisting of a mixture of Water: Acetonitrile: Acetic acid (49.5:49.5:1 v/v /v), degassed at a flow rate of 0.9 ml/min. OTA detection was performed using the wavelengths for excitation and emission, respectively. The retention time (RT) was determined to be  $\pm 0.10$  min. Samples were prepared by mixing with an extraction solution, followed by mixing and filtering. The extract was applied to the Mycosep 229 Ochratoxin column, which contains specific antibodies for OTA (monoclonal affinity chromatography). At this stage, the Ochratoxin binds to the antibody on the column, which is then washed to rid the immunoaffinity column of interferents, and by-passing methanol through the column, the Ochratoxin is removed from the antibody.

### 2.5 Validation

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the signal-to-noise (S/N) ratio of three (3:1) and ten times (10:1) of the background chromatographic noise, respectively. The different concentrations of OTA obtained by the high- performance liquid chromatography method was subjected to analysis of variance (ANOVA), and the Tukey test at a 5% level of significance ( $p=0.05$ ). The validation data is presented in Figure 1. The validation steps were carried out with samples measured against an OTA standard. They were spiked with 2.5 ng / mL OTA in three separate experiments and the recovery was 85.22%. The calibration curve and the straight equation are shown in Figure 1. The standard solutions presented a coefficient of determination ( $R^2$ ) OF 0.9979. Limit of detection of 0.125 and limit of 0.15.

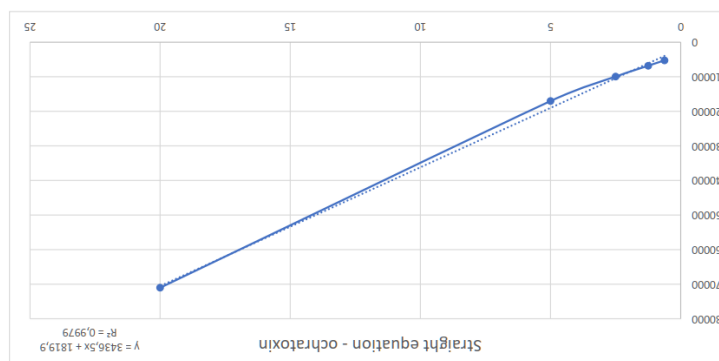


Figure 1. Calibration curve for OTA

### 3. Results and Discussion

According to the results expressed in table 2, of the 30 samples analyzed, in only 5 (16.7%) the presence of OTA was detected, above the limit of 2  $\mu\text{g}/\text{kg}$ , according to Brazilian legislation (ANVISA, 2022). In the 5 samples found with values higher than the legal limit, the ingredients in common were fruits, cereals, such as oats, beans, and rice. On the other hand, fruits and vegetables are necessary in the childhood as source of nutrients, but some of them can be contaminated to heavy metals, for example (Callen et al., 2028) and mycotoxins such as patulin in apple-based products (Zhong et al., 2018) and OTA in grapes (Li et al., 2021).

According to Andrade (2015), the foods most easily contaminated by OTA are cereals and other foods rich in starch, however, OTA has also been found in samples of coffee, spices, dried fruits, beer, and wine, and in animal meat.

Table 2. OTA level in Infant food

Samples	OTA $\mu\text{g}/\text{kg}$ <sup>1</sup>			% Samples > 2.0 $\mu\text{g}/\text{kg}$
30	Mean $\pm$ SD	min	max	16.7
	6.357 $\pm$ 8.118	0.704	25.991	

<sup>1</sup> LD= 0.125  $\mu\text{g}/\text{kg}$

In the study carried out by Cappozzo et al. (2017), it appears that 47 (30%) of 155 children's cereals were contaminated with OTA in the range of 0.6 to 22.1 ng /g. All positive samples were above the maximum level established by the European Commission (0.5 ng /g) for OTA in baby foods. OTA was detected in all types of baby cereals, but the highest incidence and concentrations were found in oat-based baby cereals (59%), followed by mixed-grain cereals (34%). In contrast, the study further indicated that organic rice-based baby foods had a higher frequency of contamination and higher concentrations of OTA than conventional rice. Regarding to the foods consumed by Brazilian children, Mallman et al. (2020), reported that mycotoxins contamination was found in 31.4% (n= 54) of the breakfast cereals and in 18.6% (n= 8) of the infant cereals.

It is therefore urgent that the awareness of food producers, combined with monitoring programs, be active in improving food storage and production conditions to prevent contamination by mycotoxins. Such actions could reduce human exposure to these compounds and prevent resulting chronic diseases. It is known that the production of mycotoxins is dependent on environmental conditions such as temperature, water activity (aw), substrate, pH, microbial interaction, among others. In this context, foods from tropical and semitropical regions may present high levels of contamination since the climate favors the development of toxigenic fungi. The consumption and production of baby foods, without due quality control and rigor, becomes more susceptible to the growth and favoring of OTA. Studies have shown that the barley grain washing process reduces the total amount of OTA in the grains by only 2-3%, thus raising concern regarding the total elimination of OTA in samples from different matrices.

To control and manage these mycotoxins, good agricultural practices (GAP), good manufacturing practices (GMP) and good storage practices (GSP) such as crop rotation, soil preparation, adequate drying, appropriate planting and harvesting time, irrigation, sanitation, and adequate storage need to be implemented. Furthermore, biotechnological approaches such as the design and production of plants that can reduce fungal growth and infection, prevent the accumulation of toxins, or resist the action of insects as a promising new pre-harvest

technique to maintain contamination levels of OTA are crucial (Kumar et al., 2020).

#### **4. Conclusion**

Samples of infant food sold in the city of Manaus-Am, Brazil were evaluated and showed the occurrence of OTA. In this context, we suggest that future work evaluate other foods intended for children and that the risk of exposure be considered for risk management and communication to health risk prevention bodies, such as health surveillance.

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#### **Authors contributions**

Sample: The sample was obtained from retail stores in the State of Amazonas. We would like to thank local merchants for their contributions to participating in this study. Dr. Kluczkovski was responsible for developing, conducting and reviewing the study. To Samir Pinto, for conducting the experimental stage, for analyzing the graphs relevant to the study and elucidating important information and to Dr. Emerson Lima for providing the equipment necessary to conduct this study. To undergraduate students Hanna Lemos and Vanderson Torres, who, together with the entire laboratory team, carried out the entire bench stage, data analysis and creation of the manuscript. All authors read and approved the final manuscript and the authors contributed equally to the study.

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#### **Competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### **Informed consent**

Obtained.

#### **Ethics approval**

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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#### **Data availability statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

#### **Data sharing statement**

No additional data are available.

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## References

- Andrade, E. D. O., Rebouças, A. S., Filho, J. Q., Ambikapathi, R., Caulfield, L. E., Lima, A. A. M., & Maciel, B. I. L. (2022). Evolution of infant feeding practices in children from 9 to 24 months, considering complementary feeding indicators and food processing: Results from the Brazilian cohort of the MAL-ED study. *Matern Child Nutr.*, *18*, e13413. <https://doi.org/10.1111/mcn.13413>
- Andrade, M., & Lances, F. M. (2015). State-of-the-art in the chromatographic analysis of Ochratoxin A in food samples. *Scientia Chromatographica*, *7*(1), 31-52. <https://doi.org/10.4322/sc.2015.015>
- Bonerba, E., Ceci, E., Balzaretto, C., Vallone, L., ... Crescenzo, G. (2017). Ochratoxin A Detection by HPLC-FL in Processed Baby Foods. *J Nephrol Ther.*, *7*, 301.
- BRAZIL (2022). NORMATIVE INSTRUCTION No. 160, of 07/01/2022. *Establishes the maximum tolerated limits (LMT) of contaminants in food.*
- Cheryl, C., Jatinder Bhatia, L.C., William, J. K., & George, M. G. (2018). Challenges and Considerations When Balancing the Risks of Contaminants with the Benefits of Fruits and Vegetables for Infants and Toddlers. *Nutrients*, *10*, 1572. <https://doi.org/10.3390/nu10111572>
- Cappozzo, J., Jackson, L., Lee, H. J., Zhou, W., Al- Taher, F., Zweigenbaum, J., & Ryu, D. (2017). Occurrence of ochratoxin A in infant foods in the United States. *Journal of food protection*, *80*(2), 251-256. <https://doi.org/10.4315/0362-028X.JFP-16-339>
- Coppa, C. F. S. C., Cirelli, A. C., Gonçalves, B. L., Barnabe, E. M. B., Petta, T., ... Oliveira, C. A. F. (2021). Mycotoxin occurrence in breast milk and exposure estimation of lactating mothers using urinary biomarkers in Sao Paulo, Brazil. *Environmental Pollution*, *279*, 116938. <https://doi.org/10.1016/j.envpol.2021.116938>
- Frey, M., Rosim, R., & Oliveira, C. (2021). Mycotoxin Co-Occurrence in Milks and Exposure Estimation: A Pilot Study in São Paulo, Brazil. *Toxins*, *13*, 507. <https://doi.org/10.3390/toxins13080507>
- Hernández, M., Juan-García, A., Moltó, J. C., Mañes, J., & Juan, C. (2021). Evaluation of Mycotoxins in Infant Breast Milk and Infant Food, Reviewing the Literature Data. *Toxins*, *13*, 535. <https://doi.org/10.3390/toxins13080535>
- Karine, V., Gilles, R., Stéphane, L., Jean-Pierre, C., & Jean-Marc, F. (2020). Dietary exposure to mycotoxins in the French infant total diet study. *Food and Chemical Toxicology*, *140*, 111301. <https://doi.org/10.1016/j.fct.2020.111301>
- Kumar, P., Mahato, D. K., Sharma, B., Borah, R., Haque, S., Mahmud, M. C., & Bui, S. (2020). Ochratoxins in food and feed: Occurrence and its impact on human health and management strategies. *Toxicon*, *187*, 151-162. <https://doi.org/10.1016/j.toxicon.2020.08.031>
- Li, X., Ma, W., Ma, Z., Zhang, Q., & Li, H. (2021). The Occurrence and Contamination Level of Ochratoxin A in Plant and Animal-Derived Food Commodities. *Molecules*, *26*, 6928. <https://doi.org/10.3390/molecules26226928>
- Mallmann, C. A., Tyska, D., Almeida, C. A. A., Oliveira, M. S., & Gressler, L. T. (2020). *Mycotoxicological monitoring of breakfast and infant cereals marketed in Brazil.* <https://doi.org/10.1016/j.ijfoodmicro.2020.108628>
- Pereira, C., Cunha, S. C., & Fernandes, J. O. (2022). Mycotoxins of Concern in Children and Infant Cereal Food at European Level: Incidence and Bioaccessibility. *Toxins*, *14*, 488. <https://doi.org/10.3390/toxins14070488>
- Rahmani, A., Jinap, S., & Soleimany, F. (2010). Validation of the procedure for the simultaneous determination of aflatoxins, ochratoxin A and zearalenone in cereals using HPLC-FLD. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, *27*, 1683-1693. <https://doi.org/10.1080/19440049.2010.514951>
- Zhong, L., Carere, J., Lu, Z., Lu, F., & Zhou, T. (2018). Patulin in Apples and Apple-Based Food Products: The Burdens and the Mitigation Strategies. *Toxins*, *10*, 475. <https://doi.org/10.3390/toxins10110475>