*Scientia Amazonia*, **v. 10, n.3, CS64-CS69, 2021**
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Ciências da Saúde

# Evaluation of Population Exposure to Aflatoxin in Mercosur: a protocol for systematic review

*Raíssa Ribeiro Monte[1], Neila Soares Picanço[2], Ariane Mendonça Kluczkovski[3], Silvânia da Conceição Furtado[4], Cleiton Mota Souza[5], Ana Cyra dos Santos Lucas[6]*

## Abstract

Aflatoxins are metabolites produced by some species of the *Aspergillus* genus, which develop naturally in food products during [the growing stages in] their production chain. The presence of these toxins in food is a factor causing considerable concern, since they are harmful to human health and affect several regions of the world, but geographically some countries are more exposed to them than others, precisely because of climatic differences. Therefore, the objective of this study is to define a systematic review protocol that assessses the risks of population exposure to these toxins, arising from both food and those originating from biological samples from countries that belong to Mercosur. Methods: Observational studies written in the languages: Portuguese, English and Spanish will be included. The search strategies will be adapted for each database used, namely: PubMed, EMBASE, LILACS, SCOPUS, Web of Science, Google Scholar, Scielo, OpenGrey and BDTD. Two reviewers will select the papers individually (in accordance with the inclusion criteria) during the two stages of the project, and the conflicts will be resolved with the help of a third reader. Subsequently, the data extracted and the risk of bias will be assessed using the Joanna Briggs Institute Prevalence Critical Appraisal Tool for studies reporting prevalence data. Conclusion: This Systematic Review is expected to produce results that may show scientific evidence of the exposure to aflatoxins of the population of Mercosur countries. This protocol was submitted to the International Prospective for Systematic Reviews (PROSPERO) and accepted in August 2020 under the registration number: CRD42020200581 (https://www.crd.york.ac.uk/PROSPERO).

**Keywords**: Mycotoxin, toxicology, food, biomarkers.

## Resumo

As aflatoxinas são metabólitos produzidos por algumas espécies do gênero *Aspergillus,* os quais se desenvolvem de forma natural em produtos alimentícios durante a cadeia produtiva deles. A presença dessas toxinas nos alimentos é um fator bastante preocupante, já que são bastante nocivas à saúde humana e acometem várias regiões do mundo, mas geograficamente alguns países se encontram mais expostos do que outros, justamente pelas diferenças climáticas. Portanto, o objetivo é descrever um protocolo de revisão sistemática que avalia os riscos de exposição tanto em alimento como também amostras biológicas oriundas de países que constituem o Mercosul. Métodos: serão incluídos estudos observacionais nas línguas: português, inglês e espanhol. As estratégias de buscas serão adaptadas para cada base de dados

[1] Undergraduate student, Faculty of Pharmaceutical Sciences, Federal University of Amazonas (FCF/UFAM). raissa_monte@hotmail.com (corresponding author)
[2] Pharmacist Researcher, FCF/UFAM, neila.nsp@gmail.com
[3] Associate Professor, FCF/UFAM, mendonca-ariane@hotmail.com
[4] Associate Professor, Department of Morphology, ICB/UFAM, silvaniafurtado@ufam.edu.br
[5] Librarian, Library System of the Federal University of Amazonas, SISTEBIB/UFAM, cleiton.mota.s@gmail.com
[6] Full Professor, FCF/UFAM, alucas@ufam.edu.br

utilizadas, sendo elas: PubMed, EMBASE, LILACS, SCOPUS, Web of Science, Google Scholar, Scielo, OpenGrey e BDTD. Dois revisores irão selecionar os trabalhos de forma individual (seguindo os critérios de inclusão) durante as duas etapas e os conflitos serão resolvidos com a ajuda de um terceiro leitor. Posteriormente, os dados serão extraídos e o risco de viés será avaliado usando a Lista de Verificação crítica de avaliação do Instituto Joanna Briggs para estudos que relatam dados de prevalência. Conclusão: Espera-se que essa Revisão Sistemática possa apresentar resultados de evidências científicas acerca da exposição das aflatoxinas na população dos países que formam o Mercosul. Este protocolo foi submetido ao International Prospective of Systematic Reviews (PROSPERO) e aceito em agosto de 2020 com o número de registro: CRD42020200581 (https://www.crd.york.ac.uk/PROSPERO).

**Palavras-chave**: Micotoxina, toxicologia, alimentos, biomarcadores.

## 1 Introduction

The mycotoxins are secondary metabolites produced by filamentous fungi of which the main representatives, are: *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria* (CHIOTTA et al., 2020). The main role of these substances is directly related to the physiological and biochemical needs of fungi to develop in the environment or, as a form of self-defense against the external threats of predators that face them (OLOMBRADA et al., 2017).

These substances come into contact with humans and animals, mainly through the ingestion of contaminated food, such as grains, oilseeds, and cereals, causing severe or chronic intoxications, depending on the quantity ingested (ARRUA et al., 2019).

In the mycotoxin group, the aflatoxins are outstanding because of their involvement in the etiology of hepatic cancer (AB et al., 2015). Their molecules are relatively stable and can remain in food throughout the entire productive chain until they reach the consumer's table (PLEADIN; FRECE; MARKOV, 2019).

Some species of the genus *Aspergillus* are the main producers of this group of toxins. The *A. flavus* produces the aflatoxin B1(AFB1) and B2 (AFB2), *A. parasiticus* and *A. nomius* produce aflatoxin G1(AFG1) and G2 (AFG2) (AC et al., 2013). These are the best-known substances that comprise this group, however, AFB1 has the highest toxigenic power. Its biotransformation in the liver can occur by means of two reactions: firstly, epoxidation, generating a compound denominated $AFB_1$-epoxide, the electrophilic characteristics of which led to their reactions through covalent bonds with DNA and RNA molecules. Secondly, hydroxylation reactions that produce AFM1, Q1, B2a, P1, and aflatoxicol (CARVAJAL, 2013).

Those products play a fundamental role as biomarkers used for evaluating the exposure to mycotoxins since these substances can in general, be found in different biological samples such as urine, blood, milk and feces (JAGER et al., 2013). AFM1 is one of the main markers excreted in urine and milk, and is useful for assessing recent exposure to AFB1, however, there are others such as the adduct AFB1-N7-guanine that is also found in the urine for a short period of time after ingesting the aflatoxins (AV et al., 2016).

Although mycotoxins occur worldwide, regions with tropical and subtropical climates are more susceptible to the aflatoxins, and consequently, the populations are more vulnerable to this contaminant in food (BENKERROUM, 2020). According to Biomin's World Mycotoxin Survey, performed in 2020,

*Scientia Amazonia*, v. 10, n.3, CS64-CS69, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

South America had a significantly high risk for mycotoxins (BIOMIN, 2021) and there are many studies that confirm this occurrence in food, as can be seen in Brazil in the study of FRANCO et al. (2019) and the human exposure to aflatoxins demonstrated by AL-JAAL et al. (2019), in some countries of this region.

Therefore, this systematic review aims to answer the guiding question: "What is the population's potential exposure to aflatoxin, considering the levels of contamination in food and human biomonitoring data, within the scope of Mercosur?".

## 2 Materials and Methods

### 2.1 Protocol and Registration

This review was registered in the International Prospective Registry for Systematic Reviews (PROSPERO) in August 2020 and registered under the number: CRD42020200581 (https://www.crd.york.ac.uk/ PROSPERO). It was conducted in accordance with the criteria defined by the Preferred Report Items for Systematic Review and Meta-Analysis Protocols, PRISMA-P (MOHER et al., 2015).

### 2.2 Eligibility Criteria

We will include observational studies from the State Parties (Argentina, Brazil, Paraguay, Uruguay, Venezuela) and Associated States (Bolivia, Chile, Colombia, Ecuador, Guyana, Peru, Surinam) of Southern Common Market (MERCOSUR). We will include studies according to the criteria established. Inclusion criteria: Studies of determination of aflatoxins in foods in Mercosul countries. Studies on human exposure to aflatoxin in Mercosul countries. Exclusion criteria: Experimental studies, animal data and toxicity studies.

### 2.3 Data sources and literature search

The search will be performed without language or time limitation until July 31, 2020, using the electronic databases PubMed, EMBASE, Scopus, LILACS, SciELO.org. We will perform an additional search in the Gray literature at Google Scholar, OpenGrey, ProQuest, and Brazilian Digital Library of Theses and Dissertations (BDTD), and manual search across the reference list of studies included.

We will use the following terms and search strategies in PubMed, which will be adapted to the others databases: (aflatoxin* OR aflatoxin b1 OR aflatoxin b2 OR aflatoxin g1 OR aflatoxin g2 OR aflatoxin m1 OR aflatoxin q1 OR TAFB1-N7-Gua OR AFB1-lysine OR aflatoxin b1-lysine OR afb1-lys OR aflatoxicol OR AFB1 OR AFB2 OR AFG1 OR AFG2 OR AFM1 OR AFM2 OR AFOH) AND (exposur* OR contaminat* OR occurrence) AND (argentina OR brazil OR brasil OR paragua* OR urugua* OR venezuela OR bolivia OR chile OR colombia OR ecuador OR equador OR guyana OR guiana OR peru OR suriname).

We will re-run searches before the final analysis. Furthermore, we will consult experts to indicate additional studies.

### 2.4 Study Selection

In the first phase of screening the studies, two authors (RRM and NSP) will screen records for inclusion, applying eligibility criteria and selecting studies for inclusion in the systematic review, independently and blinded to each other's decisions.

Disagreements between individual judgments will be resolved by consensus, and if necessary, by the judgment of a third author (ACSL). In the second phase, the two authors will assess the full texts of

*Scientia Amazonia*, v. 10, n.3, CS64-CS69, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

the remaining articles for eligibility for inclusion. The Ryyan Software (OUZZANI et al., 2016) and Zotero Version: 5.0.88.2020 (Zotero, 2020) will be used to collect references, exclude duplicates, and to perform these two phases.

### 2.5 Data extraction

For studies selected, data will be collected relative to the manuscript title, the first author (s), year of publication, study design, period, site of the study, sample size, study participants characteristics (age, sex), and outcomes of interest (aflatoxin concentration in food samples and biomarkers of concentration in of human biological samples).

Two authors (RRM and NSP) will extract the data independently and store them, using a data extraction template in a Microsoft Excel® (2013) spreadsheet. Data collected will compared, and any disagreements between reviewers will be resolved by consensus. and if necessary, discussion with a third reviewer (ACSL).

### 2.6 Risk of bias (quality) assessment

Two authors will independently assess the risk of bias by using the Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data. We will characterize the studies according to "high" when the study reaches 49% scores of "yes", "moderate" when the study reaches 50% to 69% scores of "yes" and "low" when the study reaches more than 70% scores of "yes" as suggested by Oliveira et.al. (2020).

The consensus will resolve Any disagreements between reviewers will be resolved by consensus. The funnel plot graph will be examined to check whether there is publication bias, and we will use the Egger's regression to check the symmetry of the funnel plot. Symmetric funnel plots indicate a "low risk" whilst asymmetric funnel plots indicate a "high risk" of publication bias.

### 2.7 Strategy for data synthesis

Data synthesis and statistical analysis of aflatoxin concentration in food and biomarkers in humans will be carried out by the authors. If possible, Meta-analysis will be carried out using STATA version 15. Heterogeneity between studies included will be examined using the $I^2$ statistic and p-value.

We will consider evidence of significant heterogeneity when a P-value is less than 0.10 or when we find a large $I^2$ statistic ($I^2$ 75 %). In such a case, we will perform sensitivity analysis as required. A random-effect model will be used since we may have data from widely differing population sources.

#### 2.7.1 Analysis of subgroups/subsets

We will perform sensitivity analysis/sub-group analysis based on the type of food as a source of aflatoxins, population characteristics, and region of source in Mercosur.

## 3 Discussion

The flowchart below (Figure 1) will be used to register each step of the project to be fulfilled. Furthermore, the systematic review will be conducted according to the guidelines established by PRISMA (MOHER et al., 2009).

## 4 Conclusion

By means of this systematic review, it will be expected that the population's potential exposure to aflatoxin will be evaluated, considering the levels of contamination in food, and human biomonitoring data, within the scope of Mercosur.

## Divulgation

This article is unprecedented, and it is not being considered for any other publication. The author(s) and revisors did not report any conflict of interest during its evaluation. Therefore, the magazine Scientia Amazonia owns the copyright,

has the authors' approval and permission for divulgation, of this article, by electronic means.

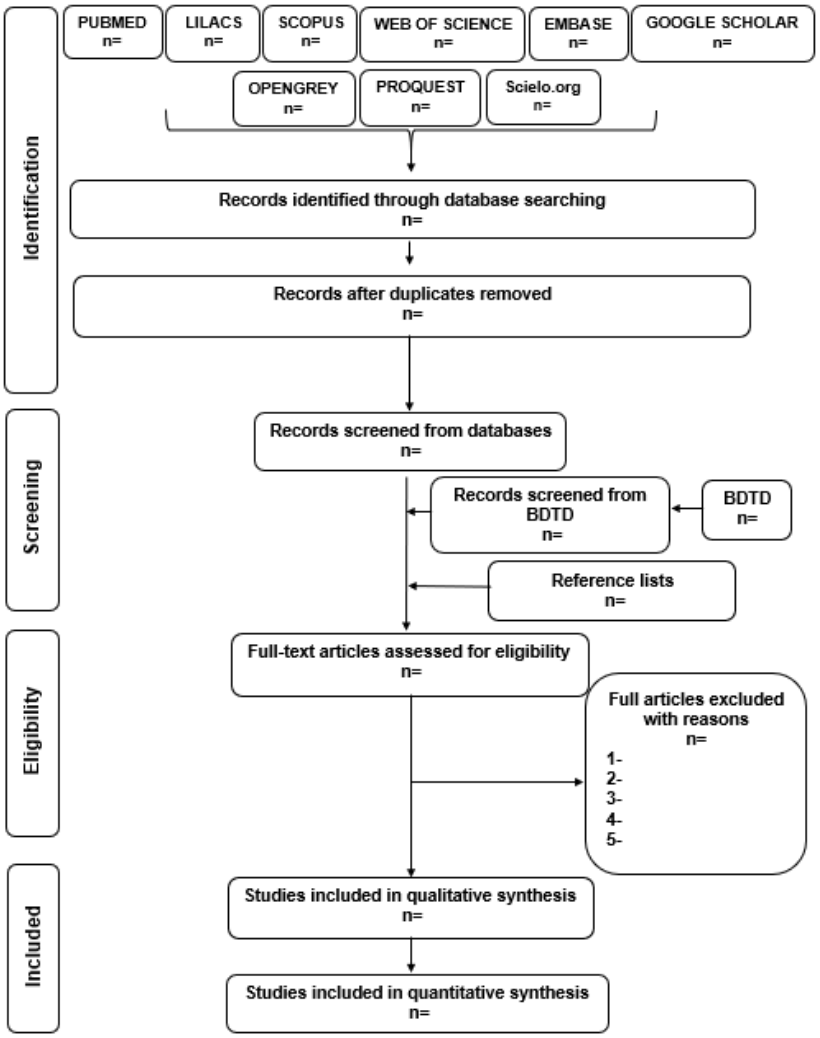


Figure 1. Flow diagram of literature search and selection criteria (adapted from PRISMA)

## References

AB, S. et al. Aflatoxin $M_1$ in raw milk from different regions of São Paulo state–Brazil. **Food additives & contaminants. Part B, Surveillance**, v. 8, n. 3, p. 207–14, 2015.

AC, B. et al. Monitoring and determination of fungi and mycotoxins in stored Brazil nuts. **Journal of food protection**, v. 76, n. 8, p. 1414–20, 2013.

AL-JAAL, B. A. et al. Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: A systematic literature review, 2001–2018. **Food and Chemical Toxicology**, v. 129, p. 211–228, jul. 2019.

ARRUA, A. et al. Presence of mycotoxins in commercially-prepared food for infants in the metropolitan area of Asuncion, Paraguay. **PEDIATRIA-ASUNCION**, v. 46, n. 2, p. 97–102, 2019.

AV, J. et al. Assessment of aflatoxin exposure using serum and urinary biomarkers in São Paulo, Brazil: A pilot study. **International journal of hygiene and environmental health**, v. 219, n. 3, p. 294–300, 2016.

BENKERROUM, N. Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. **International**

*Scientia Amazonia*, **v. 10, n.3, CS64-CS69, 2021**
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

**Journal of Environmental Research and Public Health**, v. 17, n. 2, p. 423, 8 jan. 2020.

BIOMIN. **Pesquisa Mundial de Micotoxinas: Impacto em 2021**. Disponível em: <https://www.biomin.net/br/science-hub/pesquisa-mundial-de-micotoxinas-impacto-em-2021/>. Acesso em: 8 mar. 2021.

CARVAJAL, M. Transformación de la aflatoxina B1 de alimentos, en el cancerígeno humano, aducto AFB1-ADN. **TIP**, v. 16, n. 2, p. 109–120, 2013.

CHIOTTA, M. L. et al. Toxigenic fungal species and natural occurrence of mycotoxins in crops harvested in Argentina. **Revista Argentina de Microbiología**, v. 52, n. 4, p. 339–347, out. 2020.

FRANCO, L. T. et al. Assessment of mycotoxin exposure and risk characterization using occurrence data in foods and urinary biomarkers in Brazil. **Food and Chemical Toxicology**, v. 128, p. 21–34, jun. 2019.

JAGER, A. V. et al. Assessment of aflatoxin intake in São Paulo, Brazil. **Food Control**, v. 33, n. 1, p. 87–92, 2013.

MOHER, D. et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. **PLoS medicine**, v. 6, n. 7, p. e1000097, 21 jul. 2009.

MOHER, D. et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. **Systematic Reviews**, v. 4, n. 1, p. 1, dez. 2015.

OLOMBRADA, M. et al. Fungal Ribotoxins: A Review of Potential Biotechnological Applications. **Toxins**, v. 9, n. 2, 21 fev. 2017.

OUZZANI, M. et al. Rayyan—a web and mobile app for systematic reviews. **Systematic Reviews**, v. 5, n. 1, p. 210, dez. 2016.

PLEADIN, J.; FRECE, J.; MARKOV, K. Mycotoxins in food and feed. **Advances in Food and Nutrition Research**, v. 89, p. 297–345, 2019.

**Zotero**. Fairfax, Virginia, USA: Corporation for Digital Scholarship, 2020.