Risk assessment of exposure to multiple mycotoxins in food

S. Viegas[1], C. Viegas[1], C. Ramos[1], M. Silva[1], R. Sabino[2], C. Veríssimo[2] & L. Rosado[2]
[1]*Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa (ESTeSL/IPL), Portugal*
[2]*Lab. De Micologia, Instituto Nacional de Saúde Dr. Ricardo Jorge, Portugal*

Abstract

Moulds may produce a diversity of toxins such as aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins and others. Although toxicological, environmental and epidemiological studies have addressed the problem of these toxins one by one, more than one mycotoxin are found usually in the same contaminated food. Risk assessment for humans potentially exposed to multi-mycotoxins suffers very much from the lack of adequate food consumption data. Furthermore, for a given mycotoxin, synergism and antagonism with other mycotoxins, found in the same food commodities, are not taken into account.

Aflatoxin B1 and ochratoxin A belong to the most frequently occurring mycotoxins. This has repeatedly been demonstrated, however, normally, the risk resulting from their simultaneous occurrence is not considered.

A descriptive study was developed to monitor air fungal contamination in one hospital food unit. Five air samples of 250 litres through impaction method were collected in food storage facilities, kitchen, food plating, canteen and also, outside premises, since this is the place regarded as reference.

Besides other species, *Aspergillus flavus* and *Aspergillus ochraceus* were isolated in the studied food unit. It was used weight-of-evidence scheme proposed by Mumtaz and Durkin to qualitative assess the weight of evidence for the toxicological interaction between Aflatoxin B1 and ochratoxin A.

In this case, risk assessment must be performed considering the toxicological interactions between these two mycotoxins. The limits for mycotoxins human exposure only consider the effects of each toxin and do not take into

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consideration their combined effects. Moreover, the study was developed in a setting where must be considered the sensibility of exposed population.
Keywords: exposure to mycotoxins, food contamination, multi-mycotoxins exposure, toxicological interactions, risk assessment.

1 Introduction

Assessing the toxicity and health risk of environmental chemicals is a complex process. Moreover, is well established that people are exposed to a diverse and dynamic mixture of environmental stressors as a routine part of their existence, and there is clear evidence that toxicity can be modified by simultaneous or sequential exposure to multiple environmental agents [1–3].

A major challenge in risk assessment is to determine the degree of exposure to multiple chemicals, the hazards associated with such combined exposure and the extent to which chemicals interact. Such interactions may result in effects that are either antagonistic or synergistic [4]. Risk assessments have, nevertheless, focused mainly on the narrow question of harm from exposure to individual chemicals in a specific environmental medium via a single route or pathway [5].

Chemical constituents of a mixture do not necessarily have similar properties; the composition is not necessarily constant; and the mixture may occur frequently, occasionally, or rarely. Moreover, the strength of interactive effects may differ as a function of the components doses depending on any non linearities in the dose–response relation for the biological processes affected by the components. A further complication is that the temporal characteristics of both exposures and resulting alterations in biological processes may differ for different components of coincidental mixtures [5].

Additionally, in an attempt to extrapolate from the high doses in animal studies to the lower levels to which humans are exposed, a wide range of models from simple linear extrapolation to very complex ones have been developed and used [6].

As a result, mixtures risk assessments usually involve substantial uncertainties. If the mixture is treated as a single complex substance, these doubts range from inexact descriptions of exposure to inadequate toxicity information. When viewed as a simple collection of a few component chemicals, the uncertainties include the generally poor understanding of the magnitude and nature of toxicological interactions, especially those interactions involving three or more chemicals. Because of these difficulties, the assessment of health risk from chemical mixtures must include a thorough discussion of all assumptions and the identification when possible of the major sources of uncertainty.

Based on the work developed by Mumtaz and Durkin [7] it was defined Weight-of-Evidence (WOE) classification, a judgment reflecting the quality of the available information that categorizes the most plausible nature of any potential influence of one compound on the toxicity of another compound, for a given exposure scenario. This methodology not focuses specifically on the types

of data available to support a WOE determination, but on the interpretation of the data made by an analyst or a group of analysts [7, 8].

The scheme is based on the assessment of the direction of an interaction, the plausibility that the interaction will occur, and the potential relevance of the interaction to human health.

Accordingly to this methodology four categories of confidence in the assessment are described: I - The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal; II - The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and relevance to potential human health effects is likely; III - An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak; IV - The information in this case is A. Insufficient to determine the direction of any potential interaction, B. Insufficient to determine whether any interaction would occur or C. Adequate as evidence that no toxicological interaction between/among the compounds is plausible [8].

For each category, the WOE determination is not intended to consider the magnitude of the interaction, the dose levels at which the interaction will occur, or the relative amounts of the agents in the mixture.

As described previously, the first category is intended to reflect essentially complete confidence that the interaction will occur in humans and, therefore, that interaction is assumed relevant to human health. However, a classification of Category I does not necessarily imply that the interaction has been observed in humans, or even that the interaction has been demonstrated *in vivo*. Despite this situation might often be the case, it is not strictly necessary. Nevertheless, the classification does indicate that the direction of the interaction can be predicted with confidence, and the nature of the interaction has clear toxicological relevance for humans [8].

Undesirable substances can occur in food (for example as an inherent natural constituent in the food plant or as a contaminant through their presence in the environment, through fungal contamination or through preparation processes) [6].

As secondary metabolites of toxigenic moulds, mycotoxins can represent a great risk for human health. These metabolites can contaminate the ingredients of animal feed and human food. In addition to general toxicity, their biological effects include also immunosuppressive, estrogenic and genotoxic effects.

Although toxicological, environmental and epidemiological studies have addressed the problem of these toxins one by one, more than one mycotoxin are found usually in the same contaminated food.

Aflatoxin B1 and ochratoxin A belong to the most frequently occurring mycotoxins. This has repeatedly been demonstrated but the risk resulting from their simultaneous occurrence could not be assessed due to the lack of information about their combined biological effects [9].

Aflatoxins are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans. Early evidence for the carcinogenicity of aflatoxins in humans came from descriptive studies that correlated geographic

variation in aflatoxin content of foods with geographic variation in the incidence of liver cancer (hepatocellular carcinoma, or primary liver-cell cancer). Human dietary exposure to aflatoxins at levels of nanograms to micrograms per day occurs mainly through consumption of a wide variety of contaminated crops like maize, groundnuts, cottonseed, soybeans, sorghum, rice and wheat [10].

In particularly, Aflatoxin B1 is one of the most deeply studied mycotoxins known for a long time that belongs to the group of toxins produced by the genus *Aspergillus (A. flavus, A. parasiticus, A. nominus)* [9, 11–13]. Aflatoxin B1 has been classified as a known human carcinogen by the International Agency for Research on Cancer [10].

Many authors described its hepatotoxic and hepatocarcinogenic effects in laboratory rats, mice, pigs, monkeys, ducks, trout and other animals [9, 14].

The metabolic transformation of aflatoxin B1 plays an important role in cellular activity. The most important of the metabolites, 8,9-epoxide aflatoxin B1, is formed in the organism by oxidation of cytochrome P450 monooxidases. It forms adducts with DNA and has a marked mutagenic activity confirmed by many tests carried out both *in vitro* and *in vivo* [9, 14, 15].

Ochratoxin A is the metabolite of some mould species of the genera *Aspergillus* (*A. ochraceus, A. sulphurous* and others) and *Penicillium*, especially *P. viridicatum* [16, 17]. These species of moulds grow on stored cereals, wine grapes, coffee beans, etc., and, therefore ochratoxin A can be found in a variety of food and feed and it is nearly impossible to avoid the daily exposure [17].

It is a strong nephrotoxin causing nephropathies in different species of monogastric animals, but many authors also described its hepatotoxic, teratogenic, carcinogenic and immunosuppressive effects [17–19]. Ochratoxin A inhibits proteosynthesis, mitochondrial respiration and ATP formation and increases lipid peroxidation and formation of free radicals [20, 21]. As a possible carcinogen for humans, the toxin was classified as 2B cancer compound [22, 23].

Many papers have deal with the effect of individual toxins, but the interactions between mould metabolites have been reported very rarely [9, 24, 25].

In this paper we presented a mycotoxins mixture case and tried to understand what type of interaction can occur and their potential effect on human health.

2 Materials and methods

A descriptive study was developed to monitor air fungal contamination in 10 hospital food units. Five air samples of 250 litres through impaction method were collected in food storage facilities, kitchen, food plating, canteen and, also outside premises, since this is the place regarded as reference. Considering the obtained data, we try to know toxicological relevance of the potential existence of a mycotoxins mixture in food by the employ of WOE determination.

3 Results and discussion

Besides other fungal species, in one of the analyzed food units, *Aspergillus flavus* and *Aspergillus ochraceus* were isolated and, although some strains don't produce mycotoxins, the presence of a mixture was considered. WOE determination was applied to qualitative assess the weight of evidence for the interaction between aflatoxin B1 and ochratoxin A.

Taking into consideration the categories proposed by WOE determination it is possible to conclude that Category II is applied in this interaction and potential human health effects is likely to occur. The selection of this category was based in the ability of ochratoxin A to increase the mutagenic effect of aflatoxin B1. This interaction was demonstrated in an animal model (Ames Test) in a study developed in 2001 by Sedmíková and colleagues [9]. Those results showed that the ability of ochratoxin A to increase the mutagenic effect of aflatoxin B1 may be due to the relation of these two toxins to proteosynthesis [9, 26].

Humans are most often simultaneously exposed to a large number of chemicals from different sources, and we have to take into account that combined exposures to low doses of substances that individually do not produce any adverse health effects, could still induce toxic effects when they co-occur or appear in mixtures.

Predicting risk from exposure to chemical mixtures is complex, as chemicals in mixtures can interact in terms of both toxicokinetics and toxicodynamics.

In the presented case, one important additional factor have to be considered, namely the setting where the study was performed involves higher risk because occupants vulnerability due to disease.

There is availability of a range of intervention approaches that permits prevent the adverse health effects of these common food contaminants. In this case, exposure can be avoided promoting the tight control of the food products, probably the major contamination sources.

4 Conclusions

Actually, limits for mycotoxins human exposure only consider the effects of each toxin and do not take into consideration their combined effects. It is therefore necessary to revise it considering their possible toxicological interaction and, to permit performing more accurate health risk assessments.

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