Within risk analysis, the risk assessment results are quantitative or qualitative expressions of the likelihood of harmful effects associated with exposure to a chemical. Human risk assessment of combined exposure to multiple chemicals (chemical mixtures) poses several challenges to scientists, risk assessors and risk managers, namely the complexity of the terminology and problem formulation, the diversity of chemical entities, and the toxicological profiles and exposure patterns in test species and humans.

Mycotoxins are natural contaminants produced by fungi and its frequent co-occurrence in food poses a threat to human health, mainly to vulnerable population groups as children. The MYCOMIX project (2013-15) “Exploring the toxic effects of mixtures of mycotoxins in infant food and potential health impact” aims to contribute to fill the gap concerning the risk assessment of children to multiple mycotoxins in infant foods. This project aims to answer three questions: 1) Are children exposed daily to one or several mycotoxins through food?, 2) Can this co-exposure affect children’s health? and 3) Are there interaction effects between mycotoxins?

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### Occurrence data

The occurrence data of 12 mycotoxins (aflatoxins, ochratoxin A, fumonisins and trichothecenes) were quantified in 26 Breakfast Cereals (BC), marketed in 2014 in Lisbon (PT), using HPLC-FLD, GC-MS and LC-MS/MS.

- 92% (24/26) of the analyzed BC revealed the presence of two to six mycotoxins, simultaneously, in the same sample.
- 19% of samples (4/26) were contaminated with six mycotoxins simultaneously.
- The mycotoxins mixture with highest incidence (3/26) was AFB₁, AFB₂, OTA and DON.
- Results were all below the maximum legislated limits.

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### Bioaccessibility studies

Harmonized in vitro digestion model simulating the human digestion (oral, gastric and intestinal phases) was applied to determine the mycotoxin bioaccessibility.

Bioaccessibility values for single mycotoxins ranged:
- 40 and 72% for patulin in cereal based foods.
- 86 and 104% for aflatoxin M₁ in infant formula.

Cito and genotoxicity studies (Caco-2, HK-2, HepG2)

Cytotoxicity (neutral red and MTT assays) and genotoxicity (comet assay) of mixtures involving OTA were assessed and interactions between mycotoxins were evaluated using the Concentration Addition and Independent Action Conceptual models.

- The results showed interactive toxic effects between binary mixtures of AFB₁ & OTA and OTA & FB₁, indicating that these effects should be taken into account for the hazard assessment of mycotoxins.

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### Toxicological data

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### Consumption data

Infant foods consumption data were obtained in a pilot study including 103 child (0-3 years old) from the Primary Health Care Unit of Cidada, Cascais. A 3 day food diary was applied and data introduced in a web-based platform - OPEN PORTUGAL®. For Breakfast Cereals consumption, a subsample of 75 children aged between 1 to 3 y old was used.

- 56% of children aged 1-3 years old consumed breakfast cereals at least one time in 3 days as reported in the food dairy.
- 40% of children aged 1-3 years old presented a mean weight of 13.39 kg and a mean consumption of breakfast cereals of 5.62 g day⁻¹.

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### Exposure assessment data

Two mathematical approaches: deterministic and probabilistic (Monte Carlo simulation) will be used for the computation of the exposure assessment for mycotoxins.

Different exposure scenarios for the mycotoxin dietary exposure assessment in relation to the data treatment of the non-detects (< LOD, limit of detection) will be included.

- Daily exposure of children to ochratoxin A, fumonisins and trichothecenes showed no health risks to the children population considering individual mycotoxins (hazard quotients below 1).5

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