

# Evaluation of masked mycotoxins in food and their release and uptake in the gut

Maes o ddiddordeb ymchwil: <u>Chemical hazards in food and feed</u> Hyd yr astudiaeth: 2014-01-01 Cod prosiect: FS102101 Cynhaliwyd gan: Food and Environment Research Agency (now Fera Science Ltd)

# Background

Mycotoxins are a group of naturally occurring chemicals produced by certain moulds. Masked mycotoxins are metabolites of the parent mycotoxin formed in the plant or fungus as a result of plant defence reactions after fungal infection or are produced by the fungus itself. Masked mycotoxins can be released, hydrolysed, biotransformed and absorbed in the human gastrointestinal tract, primarily as the parent compound. The levels at which these masked forms are present in food and their likely behaviour in the human gut was examined in this study.

In 2014, the European Food Safety Authority published (EFSA) published its Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. EFSA is currently assessing the risks for human and animal health related to the presence presence of deoxynivalenol, metabolites of deoxynivalenol and masked deoxynivalenol in food and feed. Data from this study were submitted to EFSA for inclusion to their ongoing exposure assessments.

# **Research Approach**

### Objectives

To undertake a survey investigating the presence of masked mycotoxins in retail food samples from the UK market, and to conduct in vitro studies of the metabolism and transport of certain masked mycotoxins in the human gut. These experiments will lead to a fuller understanding of the possible significance of masked mycotoxins in the diet.

#### **Our Research Approach**

Survey of UK retail products for masked mycotoxins

184 UK retail food samples consisting of cereal products (25), breakfast cereals (60), cerealbased infant foods (30), beer (30) and spices (39) were analysed using a highly sensitive analytical method based on liquid-chromatography with tandem mass spectrometry (LC-MS/MS) for the presence for mycotoxins - both the parent compounds (deoxynivalenol, T-2 toxin, HT-2 toxin, nivalenol and zearalenone) and their masked forms (3-Acetyl-deoxynivalenol,15-Acetyldeoxynivalenol, deoxynivalenol-3-glucoside, ?- and ?-zearalenol, ?- and ?-zearalenol-14glucoside, zearalenone-14-glucoside and Zearalenone-14-sulphate). Investigation on release and uptake of parent mycotoxin from the masked form in the gut

Small intestinal metabolism was studied by exposing masked mycotoxins (deoxynivalenol-3glucoside T2-3-glucoside, zearalenone-14-glucoside, ? and ? zearalenol-14-glucoside) and the respective parent mycotoxins with artificial digestive juice to mimic the upper gastrointestinal conditions. The large intestinal metabolism these compounds were studied using faecal batch cultures from 5 donors. Finally an in vitro study of the gut transport of masked mycotoxins and parent compounds was conducted using human intestinal epithelial cell line Caco-2 clone TC7.

# Results

Masked mycotoxins were detected in 19% of the samples at low levels. *In vitro* studies with certain masked mycotoxins showed that while masked mycotoxins are not hydrolysed by small intestinal digestive juices, they are efficiently hydrolysed by faecal microbiota. The parent mycotoxins are absorbed by the intestinal epithelium, but the masked forms are not. The data generated from this survey has been submitted to EFSA to contribute to their risk assessments.

#### Survey of UK retail products for masked mycotoxins

None of the samples analysed contained mycotoxins (deoxynivalenol, T-2 toxin, HT-2 toxin, nivalenol and zearalenone) at or above the EU maximum permitted levels set out in Commission Regulation (EC) No 1881/2006 (as amended).

Masked mycotoxins were detected in 35 samples with levels ranging from 3.8 - 121  $\mu$ g/kg, the highest level was found in a sample of breakfast cereal.

Deoxynivalenol-3-glucoside (masked mycotoxin) was detected in 31 samples of beer, breakfast cereal, cereal products, cereal based infant foods and spices, with levels ranging from 20 - 121  $\mu$ g/kg. ? -zearalenol was found in a sample of curry powder at 3.8  $\mu$ g/kg, and ?-zearalenol-14-glucoside at 13  $\mu$ g/kg in a sample of ground cumin. Zearalenone-14-glucoside and Zearalenone-14-sulphate were each detected in two different spice samples at levels ranging from 10-18  $\mu$ g/kg.

#### Investigation on release and uptake of parent mycotoxin from the masked form in the gut

All masked mycotoxins tested were stable under incubation conditions mimicking the small intestinal digestion and no hydrolysis was observed. Human gut microbiota efficiently hydrolysed all masked mycotoxins suggesting their efficient hydrolysis in the large intestine. Deoxynivalenol-3-glucoside and T2-3-glucoside were fully recovered as parent mycotoxins whereas 40-70% of zearalenone compounds were further metabolized to unknown metabolites. None of the masked mycotoxins tested were efficiently hydrolysed or transported through the epithelial cell layer while the free parent mycotoxins were transported.

The study has shown some interesting results and also raised some questions. The results of the faecal incubation studies show that through microbial activity, modified mycotoxins can be hydrolysed to the parent or free mycotoxins. It is therefore possible that micro-organisms residing in the upper gut could also hydrolyse masked mycotoxins releasing free mycotoxins that could add further to the mycotoxin exposure of individuals. However further studies using animal models would be required to assess this.

## **Published Papers**

 Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota in vitro. Gratz SW, Dinesh R, Yoshinari T, Holtrop G, Richardson AJ, Duncan G, MacDonald S, Lloyd A, Tarbin J. Mol Nutr Food Res. February 2017.

Research report

#### England, Northern Ireland and Wales

PDF

Gweld Evaluation of Masked Mycotoxins in Food and Their Release and Uptake in the Gut as PDF(Open in a new window) (2.71 MB)