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Endocrine activity of mycotoxins and mycotoxin mixtures

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ABSTRACT

Reporter gene assays incorporating nuclear receptors (estrogen, androgen, thyroid β and PPARγ2) have been implemented to assess the endocrine activity of 13 mycotoxins and their mixtures.

As expected, zearalenone and its metabolites α-zearalenol and β- zearalenol turned out to have the strongest estrogenic potency (EC50 8,7 10-10 ± 0,8; 3,1 10-11 ± 0,5 and 1,3 10-8 ± 0,3 M respectively). The metabolite of deoxynivalenol, 3-acetyl-deoxynivalenol also had estrogenic activity (EC50 3,8 10-7 ± 1,1 M). Furthermore, most of the mycotoxins (and their mixtures) showed anti-androgenic effects (15-acetyldeoxynivalenol, 3-acetyl-deoxynivalenol and α-zearalenol with potencies within one order of magnitude of that of the reference compound flutamide). In particular, deoxynivalenol and 15-acetyl-deoxynivalenol acted as antagonists for the PPARγ2 receptor.

When testing mixtures of mycotoxins on the same cell systems, we showed that most of the mixtures reacted as predicted by the concentration addition (CA) theory. Generally, the CA was within the 95% confidence interval of the observed ones, only minor deviations were detected.

Although these reporter gene tests cannot be directly extrapolated *in vivo*, they can be the basis for further research. Especially the additive effects of ZEN and its metabolites are of importance and could have repercussions *in vivo*.

1. Introduction

A lot of substances in food and feed, can interfere with the hormonal system of humans and animals by influencing the synthesis, metabolism or transport of hormones; or by interacting directly with the hormone receptor (as an agonist or antagonist) (Wuttke et al., 2010; Diamanti-Kandarakis et al., 2009). Over the past decades there has been a growing concern about the exposure of animals and humans to these endocrine disrupting chemicals (EDCs). EDCs can be chemicals of synthetic (pesticides, industrial chemicals, bisphenols) or natural origin (mycotoxins, phytoestrogens) (Diamanti-Kandarakis et al., 2009).

Mycotoxins are natural components that can cause adverse health effects in an organism. These toxins result from the secondary metabolism of moulds and are mainly produced by 6 genera of *fungi: Aspergillus, Penicillium, Fusarium, Alternaria, Claviceps* and *Stachybotrys* occurring in food and feed commodities both pre- and post-harvest (Milicevic et al., 2010). A substantial part of the crops that are grown in the EU for food and feed contain a detectable amount of mycotoxins (Streit et al., 2013; Marin et al., 2013). The adverse effects include carcinogenic, mutagenic, estrogenic, nephrotoxic, neurotoxic, hepatotoxic, immunosuppressive and gastrointestinal toxicity (Milicevic et al., 2010; Fung and Clark, 2004). The potential health risks of mycotoxins require legal limits of maximal permissible concentrations in food and feed, based on toxicological and/or epidemiological data. For a significant number of mycotoxins and a limited number of combinations (aflatoxins, fumonisins) the maximal levels have already been determined (in EU, maximal limits are defined in regulation (EC) n°1881/2006 for food and guidelines in Directive 2002/32/EC, for feed).

Results also indicate that certain mycotoxins and their metabolites can act as potential endocrine disrupters at the level of nuclear receptor signalling and cause a change in hormone production. In this context, zearalenone (ZEN) and its metabolites

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Abbreviation

3-acetylDON	3-acetyl-deoxynivalenol
15- acetylDON	15-acetyl-deoxynivalenol
α-ZEL	α-zearalenol
ALT	alternariol
AR	androgen receptor
β-ZEL	β-zearalenol
CA	concentration addition
CALUX	Chemically Actived LUciferase eXpression
CIT	citrinin
DBP	dibutylphthalate
DHT	dihydrotestosteron
DMSO	dimethylsulfoxide
DON	deoxynivalenol
ER	estrogen receptor
FB1	fumonisin B1
FB2	fumonisin B1
FUSx	fusarenon x
IARC	International Agency for Research on Cancer
NIV	nivalenol
OTA	ochratoxin A
PBS	phosphate buffered saline
PPARγ2	peroxisome proliferator-activated receptor type 2
ROS	rosiglitazone
SD	standard deviation
SEM	standard error of the mean
T3	triiodothyronine
TDI	tolerable daily intake
TRβ	thyroid receptor β
ZEN	zearalenone

α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) are estrogens (Frizzell et al., 2011; Tiemann and Danicke, 2007; Molina-Molina et al., 2014; Cortinovis et al., 2013). *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium heterosporum* are ZEN producing species. The risk of contamination is highest in cereal crops but silages and straw are also likely to contain ZEN (Marin et al., 2013; Streit et al., 2012). ZEN is rapidly absorbed and metabolized *in vivo* to α-ZEL and β-ZEL. So, mixtures of them may be present in biological systems (Zinedine et al., 2007). α-ZEL exhibits the strongest estrogenic potency (comparable to 17β-estradiol) (Marin et al., 2013; Frizzell et al., 2011; Molina-Molina et al., 2014). Moreover, these mycotoxins increase the production of progesterone, estradiol, testosterone and cortisol hormones in the H295R steroidogenesis assay (Frizzell et al., 2011). *In vivo*, ZEN and its metabolites, α-ZEL and β-ZEL, exhibit endocrine activity in pigs, bovine and sheep causing infertility problems (Tiemann and Danicke, 2007; Cortinovis et al., 2013).

The toxicity of type B trichothecenes such as deoxynivalenol (DON), nivalenol (NIV) and fusarenon x (FUSx, the precursor of nivalenol) involve inhibition of protein and DNA synthesis and general cytotoxicity (Fung and Clark, 2004). DON is mainly produced by *F. culmorum* and *F. graminearum*. DON contamination is observed worldwide, with cereal crops such as wheat, maize or barley being most frequently affected. Furthermore, silage contamination is regularly observed (Marin et al., 2013). DON may be produced together with its acetylated derivatives, 3-AcetylDON and 15-AcetylDON. DON is considered to be responsible for nausea and decreased feed intake in pigs and is an immunosuppressant (Marin et al., 2013). *In vitro*, there is no evidence that DON can interact directly with steroid hormone receptors to cause endocrine activity, but effects on steroidogenesis and alterations in gene expression indicate its potential as an endocrine disrupter (Ndossi et al., 2012). Nivalenol is a mycotoxin produced by *Fusarium cerealis* and *Fusarium poae* and to a lesser extent also *F. culmorum* and *F. graminearum.* These fungi are abundant in various cereal crops (wheat, maize, barley, oats, and rye).

Fumonisins are mycotoxins that are mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum* found in maize and maize-products with a strong similarity to sphinganine, the backbone precursor of sphingolipids. They inhibit ceramide synthase, a critical enzyme for the synthesis of sphingolipids. Fumonisins are involved in acute and chronic toxicity in several animal species, including carcinogenicity (Marin et al., 2013). The International Agency for Research on Cancer (IARC) has classified fumonisin B1 (FB1) as possibly carcinogenic to humans (Group 2B). Interestingly, sphinganine was shown to bind receptors of the PPAR family *in vitro* (Van Veldhoven et al., 2000).

Alternariol (ALT) is produced by *Alternaria alternata*, and occurs e.g. on cereals, sunflower seeds, oilseed rape, olives and various fruits. It causes toxic effects in animals, including fetotoxic and teratogenic effects. ALT is mutagenic and clastogenic in various *in vitro* systems (Marin et al., 2013). ALT also exhibits a weak estrogenic response and results in a significant increase in estradiol and progesterone production (in H295R cells) (Frizzell et al., 2013a; Lehmann et al., 2006).

In temperate regions, OTA contamination is mostly due to *Penicillium verrucosum* infection while *Aspergillus* species such as *Ascobolus carbonarius* account for OTA production in warmer regions. As for feed ingredients, OTA is most frequently found in cereals but is also known to contaminate soy beans and peanuts (Streit et al., 2012). OTA inhibits protein, DNA and RNA synthesis. OTA has been shown to induce many toxic effects in animals, the most prominent being nephrotoxicity; it is also classified as possibly carcinogenic to humans (IARC, Group 2B) (Marin et al., 2013). *In vitro*, OTA also shows some endocrine activities, it may be a mild antagonist for the estrogen, androgen, progestogen and glucocorticoid receptor and increases the production of estradiol in H295R cells (Frizzell et al., 2013b). Citrinin (CIT) is also produced by several species of *Aspergillus* and *Penicillium* (including *P. verrucosum).* CIT is less stable then OTA, it appears to be destroyed by food processing and thus could be more a problem for life stocks then for humans, it mostly acts as a nephrotoxin (Fung and Clark, 2004). However, recently it was found that CIT (and its metabolite dihydrocitrinone) was present in 90% of human urine samples which means that humans are much more exposed to CIT than realized before (Huybrechts et al., 2014).

The information mentioned above illustrates that mycotoxigenic fungi are usually capable of producing more than one mycotoxin and that feed or food raw materials are commonly infected with various fungal species or strains at a time. Hence, studying the occurrence of any given mycotoxin alone provides incomplete information about the risk associated with it (Streit et al., 2012). Streit and co-workers provide an overview of co-occurrence of several mycotoxins in feed in Europe. Typically, DON was found to be the major contaminant, frequently co-occurring with ZEN, as for OTA and CIT (Streit et al., 2012).

Both *in vivo* and *in vitro* additive, synergistic or antagonistic effects are detected for mixtures of mycotoxins (Alassane-Kpembi