

Review Article

Mycotoxins and Nuclear Receptors: A Still Underexplored Issue

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Abstract. Mycotoxins are fungal secondary metabolites that can be found in food commodities worldwide. They exert a wide range of adverse effects towards humans and animals. Although toxicological studies have addressed these food contaminants over decades, their mode of actions as well as their synergistic effects are still to be deeply clarified. Among the toxicological targets, nuclear receptors have been identified by several studies. Besides the estrogenic effect, a wider range of endocrine and neuroendocrine disrupting effects have been reported so far. This review is aimed at addressing the recent advances in toxicology, and at highlighting possible gaps of knowledge.

Keywords: Endocrine disruptors, aflatoxins, deoxynivalenol, natural toxins

1. Mycotoxins in Food Commodities

Mycotoxins are a group of naturally occurring low molecular weight secondary metabolites produced by pathogenic fungi in the field. They can grow on a variety of different crops including cereals, nuts, spices, dried fruits, oil seeds, cocoa and coffee, as reported in Table 1.

Mycotoxins occur more frequently in areas with a hot and humid climate, favorable for the growth of pathogenic fungi on field crops [1, 2]. Drought and warm temperature are considered as supportive climatic conditions for fungal infection in the fields [3, 4]. As a consequence, the climate change is affecting significantly the pathogen infection in crops and the subsequent accumulation of mycotoxins in the

field worldwide, even in more temperate areas. Mycotoxins may also accumulate in crops postharvest under uncontrolled storage conditions [5, 6].

On account of the large differences in chemical structures among mycotoxins, the spectrum of biological functions exerted by these compounds is actually very broad. Besides giving the molds a competitive advantage over other mold species and bacteria, mycotoxins may act as virulence factors for plants. In both cases, biological mechanisms are still to be clarified.

Currently, more than 300 mycotoxins are known, but scientific attention is focused mainly on those that have proven to be a health hazard at levels commonly occurring in crops [6]. From a toxicological point of view, mycotoxins

can cause a variety of adverse health effects in humans and animals (see Table 1). For most of these compounds, a tolerable daily intake (TDI) has been established, and appropriate regulation and guidelines have been set in many countries worldwide [3, 6].

Diet is the main route of exposure for humans and animals, although toxicoses induced by air and dust containing toxins have been reported [7, 8]. Besides direct contamination of grains and plants commodities, humans can be exposed through carry over from animal products such as milk, meat, and eggs [6, 9, 10].

Mycotoxins are nearly all cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis [11].

In 1993, the WHO-International Agency for Research on Cancer evaluated the carcinogenic potential of aflatoxins, ochratoxin A, trichothecenes, zearalenone, and fumonisins. Among them, aflatoxins were classified as carcinogenic to humans (Group 1) while ochratoxin A and fumonisins were classified as possible carcinogens (Group 2B). Trichothecenes and zearalenone were not classified as human carcinogens (Group 3), as they exert their activity through different mechanisms [12, 13]. Nonetheless, recent studies pinpointed that the toxicological and carcinogenic related effects of mycotoxins remain to be understood [14].

Among the mycotoxins of most concern for mammals, aflatoxins are known to be potent hepatocarcinogenic and genotoxic compounds [11]. Other mycotoxins, such as ochratoxin A, patulin, and *Fusarium* toxins (i.e. trichothecenes, fumonisins, and zearalenone) have a range of other health effects including kidney damage, gastrointestinal disturbances, reproductive disorders or suppression of the immune system [11, 15].

Mycotoxins have significant economic impacts in numerous crops, especially cereals and nuts, cottonseed, and coffee. The Food and Agriculture Organization has estimated that 25% of the world's crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tons of foods and food products (<ftp://ftp.fao.org/esn/food/myco4a.pdf>). Economic losses are caused by the yield loss of food/feed commodities as well as by the reduced crop values in contaminated batches. Furthermore, human health and veterinary costs are relevant. Additional costs include the cost of management at all levels—prevention, sampling, mitigation, legislation, discharge of non-compliant batches.

Although it is clear that mycotoxins affect human health, especially in developing countries, their impact is often difficult to quantify, as they can induce acute toxicoses and immunosuppression, as well as chronic effects. In this framework, epidemiological studies may support the research, although often limited to population groups located in developing countries, where exposure to mycotoxins can

even be extremely high. Outbreaks of aflatoxicosis in Sub-Saharan Africa have led to hundreds of fatalities, even recent years [16, 17]. On the other side, frequent outbreaks of aflatoxins in Europe over the last decade have mainly shown their impact on animal health, with serious implication on milk and dairy sector in Mediterranean and Balkan area [18, 19].

2. Adverse Effects of Mycotoxins on Humans

Mycotoxins have a broad spectrum of adverse effects, including hepatotoxicity, estrogenicity, immuno/haematotoxicity, nephrotoxicity or neurotoxicity. Some of them are recognized as genotoxic and/or carcinogenic. The main effects related to the regulated mycotoxins are reported in Table 2.

Mycotoxicoses can be classified as acute, with a rapid onset and a clear toxic response, or chronic, when a low-dose exposure over a long time period leads to generally irreversible effects [20]. Over the centuries, fungal pathogens have been implicated in several human outbreaks of mycotoxicoses. As an example, ergotism due to the ingestion of ergot alkaloids from *Claviceps purpurea* in rye is known since Middle Age. More recently, cereal grains contaminated with *Fusarium sporitrichoides* and *F. poae* were implicated in alimentary toxic aleukia in Russia in '30s and '40s [21].

Among mycotoxicosis, those related to aflatoxins and fumonisins exposure are of major concern in developing countries. Repetitive aflatoxin outbreaks have indeed occurred in recent years in Kenya, India, and Malaysia, often in association with fumonisins or other mycotoxins [21, 22]. In 2004, 125 people died following a major outbreak of aflatoxicosis in the eastern and central provinces of Kenya. Three hundred and seventeen cases were reported, and most were linked to aflatoxin poisoning from contaminated maize.

It must be mentioned that Aflatoxin B1 (AFB1) is a primary cause of human liver cancer, and in developing countries it acts synergistically with the Hepatitis B virus (HBV) infection [23]. It is reported that approximately 250,000 deaths are caused by hepatocellular carcinomas in China and Sub-Saharan Africa annually. These diseases are attributed to risk factors such as high daily intake of aflatoxins and high incidence of hepatitis B [24]. Moreover, mycotoxins are often related to the impairment of children's growth [14, 25, 26]. Very recently, it has been shown that insulin-like growth factor 1 (IGF-1) axis may represent a common causal pathway in mycotoxin effects on hepatocellular carcinoma as well as growth retardation [27].

Biomarkers' studies showed that aflatoxin exposure is linked to other disease such as Kwashiorkor and Reye's syndrome, as well. Furthermore, aflatoxins seem to exert a modulating effect in cases of zinc, iron and vitamin A deficiency animals, thus suggesting a similar role in humans [28].

Table 1: Main mycotoxins and their toxic effects in humans.

Mycotoxins	Commodities	Main associated fungi
Aflatoxins	Maize, peanuts, tree nuts, dried fruits, spices	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Fumonisin	Maize	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>
Ochratoxin A	Cereals, dried fruits, grapes and wine, coffee, cocoa	<i>Aspergillus ochraceus</i> , <i>Penicillium verrucosum</i> , <i>P. nordicum</i>
Patulin	Apples, grapes, other fruits	<i>Penicillium spp.</i>
Deoxynivalenol	Maize, wheat, barley, oats, beer	<i>Fusarium graminearum</i> , <i>F. culmorum</i>
Zearalenone	Maize, wheat, barley, oats, beer	<i>Fusarium graminearum</i> , <i>F. culmorum</i>

Table 2: Mycotoxins and main associated adverse effects.

Mycotoxins	Effects	Cellular and molecular mechanisms of action
Aflatoxin B1 and M1	Hepatotoxicity Genotoxicity Carcinogenicity Immunomodulation	Formation of DNA adducts Lipid peroxidation Bioactivation by cytochromes P450 Conjugation to GS-transferases
Fumonisin	Central nervous system damage Hepatotoxicity Genotoxicity Immunomodulation	Inhibition of ceramide synthesis Adverse effect on the sphinganine/sphingosin ratio Adverse effects on the cell cycle.
Ochratoxin A	Nephrotoxicity Genotoxicity Immunomodulation	Effect on protein synthesis. Inhibition of ATP production Detoxification by peptidases
Patulin	Neurotoxicity <i>In vitro</i> mutagenesis	Indirect enzyme inhibition
Trichothecenes (i.e. DON, T-2, HT-2)	Hematotoxicity Immunomodulation Skin toxicity	Induction of apoptosis in haemopoietic progenitor cells and immune cells. Effect on protein synthesis Abnormal changes to immunoglobulins
Zearalenone	Reproductive adverse effects	Binding to oestrogen receptors Bioactivation by reductases Conjugation to glucuronyltransferases

Human aflatoxicosis in developing countries is often associated to exposure to fumonisins, that are possible carcinogen compounds in humans [29]. Fumonisin also inhibit the uptake of folic acid via the folate receptor, and have been implicated in the high incidence of neural tube defects in rural populations [30].

Among other mycotoxins, ochratoxin A has been shown to be nephrotoxic, immunosuppressive, carcinogenic and teratogenic. It is often related to renal disease, both in human and animals.

Porcine nephropathy caused by OTA exposure in pigs and balkanic endemic nephropathy (BEN) in humans present similarities in morphological and functional kidney lesions in endemic nephropathy: For this reason, OTA has been proposed as one of the causative agents for BEN, a fatal renal disease occurring among rural populations in the Balkan area [31]. Although the causative relation is still to be proven, a significantly higher exposure to OTA was proven for

inhabitants from endemic regions than control regions [32]. However, further studies are need to better understand the role of ochratoxin A exposure in renal diseases [33, 34].

Trichothecenes, mainly DON and T-2 and HT-2 toxins, are known as immunosuppressive agents, and can cause vomiting and food refusal. In 1931 extremely high levels of T2 and HT2 in stored grains caused an outbreak of alimentary toxic aleukia (ATA) syndrome in the USSR, with a mortality rate of 60% [35]. Nonetheless, that was the only outbreak of human mycotoxicosis related to trichothecenes ingestion, while more chronic and sub-chronic effects are often related to intestinal disease [36]. Concerning zearalenone and its metabolites, their role as endocrine disruptor is well-known and often related to estrogenic effects in humans [37, 38].

In consideration of the immunosuppressive activity and the broad spectrum of toxic activities, mycotoxins might be implicated in a large number of human diseases, although the lack of adequate epidemiological studies, the few information

on the mode of action and the presence of several confounding factors make difficult to set clear cause-effect interaction [14].

3. Mycotoxin and Nuclear Receptors

Chronic toxic effects such as carcinogenesis, genotoxicity, mutagenicity, and immunosuppression have been studied so far as a consequence of mycotoxin exposure. Nonetheless, several recent studies describe the interaction of these compounds with nuclear receptors at different levels. Besides the mycoestrogen zearalenone, whose estrogenic adverse effects are due to its high binding affinity to the estrogen receptor, other mycotoxins have been described as able to disrupt the biological function of nuclear receptors at various levels. The possible interaction reported in the literature so far have been summarized in Table 2.

3.1. Zearalenone. Zearalenone (ZEN) is a non-steroid estrogen mycotoxin produced by numerous strains of *Fusarium* which commonly contaminate cereals (Figure 1)

It is known that ZEN cause hormonal effects in animals, such as abnormalities in the development of the reproductive tract and mammary gland in female offspring, suggesting a fetal exposure to these contaminants.

After ingestion, zearalenone undergoes reductive phase I metabolism to form α - and β -zearalenol (α - and β -ZEL), zearalanone (ZAN), and α - and β -zearalanol (α - and β -ZAL).

Zearalenone binds to ERs in vitro with similar affinity for both forms of estrogen receptor, ER α and ER β [31]. Among ZEN metabolites, α -ZEL showed the highest affinity for ERs (-32, 33). Accordingly, animal species that mainly metabolize ZEN into α - form are those commonly showing the highest sensitivity. Similarly, the specie-specific sensitivity can be explained taking into consideration the binding affinity towards specie-specific forms of the estrogen receptor as well as the ratio between ER α and ER β [39–41]. Unfortunately, extensive comparative studies are still lacking.

Very recently, the mechanism of binding has been elucidated by molecular modelling studies [42]. This approach was further exploited to predict and rank ZEN metabolites towards ER α for hazard characterization [43, 44]. The investigation of receptor-ligand interactions through docking simulation proved to accurately rank estrogenic potencies of ZEN and its metabolites, showing the suitability of the model to address estrogenic potency for this group of compounds [43, 44].

While the interaction of zearalenone and its modified forms to ER α has been fully characterized in humans and animals, much less is known about the possible interaction of these compounds with other nuclear receptors.

A recent study investigated the possible interaction between ZEN and its metabolites with the human androgen

receptor (hAR) [41]. In particular, their ability to induce hAR-mediated reporter gene expression was examined in androgen-sensitive PALM cells. Results demonstrated that the tested compounds showed hAR-mediated antagonistic activity in PALM cells. In particular, the metabolite ZAN and its reduced forms α - and β -ZAL were the most effective hAR antagonists.

By comparing the effects in vitro on human trophoblast (BeWo cells), α -ZEL and β -ZEL have been found to not affect cell differentiation, unlikely their parent compound. In addition, the reduced forms induced significant changes in ATP-binding cassette (ABC) transporter expression, and this was explained on the basis of potential interaction with nuclear receptors (liver X receptor LXR, pregnane X receptor PXR, progesterone receptor PR) that could modify the transport function of placental cells [45]. The same authors showed that the mechanism of differentiation induction exerted by ZEN in BeWo cells does not involved peroxisome proliferator-activated receptor γ (PPAR γ).

These results suggested that the metabolic biotransformation of ZEN may lead to interferences with the endocrine system by different modes of action, acting as exogenous endocrine disruptors in mammals at various molecular levels.

3.2. Toxins from *Alternaria*. *Alternaria* toxins are a group of mycotoxins produced by *Alternaria* spp. on grains and fruits [46]. Although they are often reported to interact with nuclear receptors, mainly as endocrine disruptors [47, 48], their main toxic effect is related to DNA strand breaking activity [49]. In vivo effects are on the other side related to the lung and oesophageal cancer in human [50]. Chemical structures of AOH and its main derivatives are reported in Figure 2.

According to in vitro studies, alternariol (AOH) may replace estradiol from isolated human estrogen receptors α and β in a human endometrial adenocarcinoma cell line (Ishikawa cells). The estrogenicity of AOH was about 0.01% of that of natural ligand estradiol [48].

The AOH endocrine activity of was recently studies in mammalian cell lines, using a reporter gene assay that incorporate natural steroid hormone receptors for oestrogens, androgens, progestagens and glucocorticoids [51]. The activity was indeed studied at the level of nuclear receptor transcriptional activity. Alternariol exhibited a weak oestrogenic response, and it synergistically increased the binding of progesterone to the progestagen receptor. While highest concentrations of AOH did not significantly affect testosterone and cortisol hormones, estradiol and progesterone production were strongly increased. According to the authors, AOH showed a weak oestrogenic activity, but was able to interfere with the steroidogenesis pathway [51]. Anyway, little to nothing is known about the effects of biotransformation on AOH and other structurally related mycotoxins form *Alternaria*. Since AOH undergoes metabolic pathways as already described for ZEN, the estrogenic potential of metabolites and their ability to bind the estrogen receptor

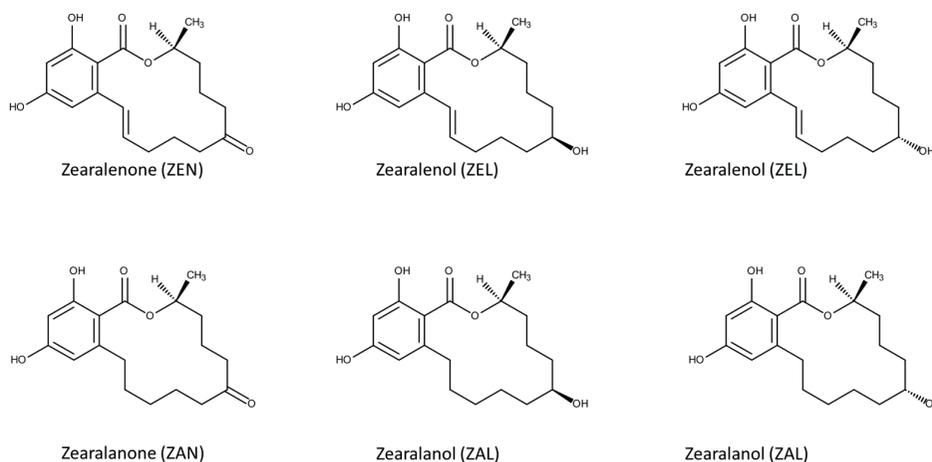


Figure 1: Structure of zearalenone and its main metabolites.

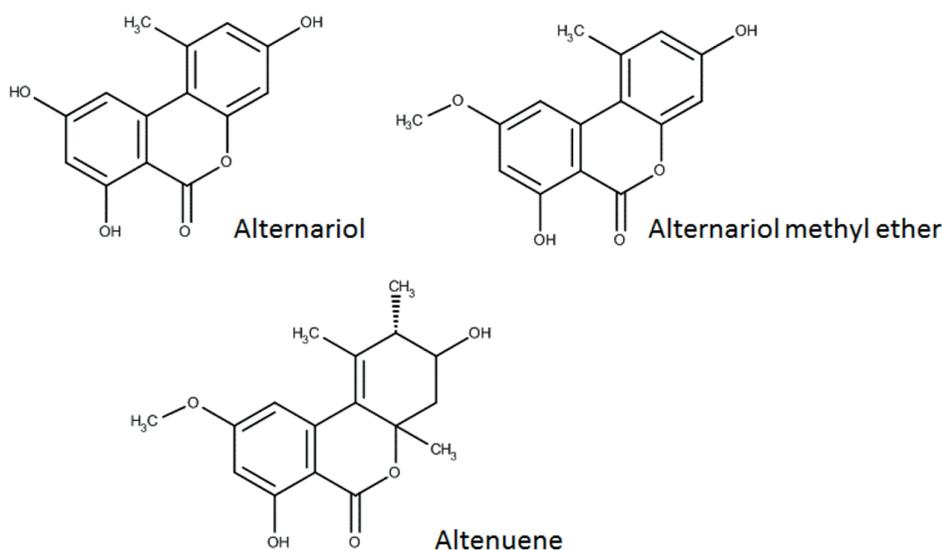


Figure 2: Structure of main mycotoxins from *Alternaria* spp.

in different animal species should be considered in further studies.

Besides estrogen receptor, mycotoxins from *Alternaria* spp are able to interact with other nuclear receptors. Alternariol and its methyl ether derivative were indeed reported to be able to induce the aryl hydrocarbon receptor complex AhR/ARNT pathway in murine hepatoma cells, mediating the induction of cytochrome P450 1A1 enzyme (CYP1A1) and apoptosis [52]. Nonetheless, the mechanism of action has still to be investigated in details.

3.3. Aflatoxins and trichothecenes: endocrine and neuroendocrine effects. Trichothecene mycotoxins such as deoxynivalenol (DON), T-2 toxin (T2), and HT-2 toxin (HT2) (see

Figure 3) are known to inhibit eukaryotic translation and to trigger the ribotoxic stress response, which regulates gene expression via the activation of the mitogen-activated protein (MAP) kinase superfamily. Although the interaction between DON and T2 with the 60S peptidyl transferase centre of the eukaryotic ribosome, described by Garreau de Loubresse et al. [53] and related to the ribotoxic stress response, is commonly considered as responsible for the broad spectrum of toxic activity exerted by this compound, little is known on the possible interaction with nuclear receptors.

In human lung carcinoma A549 cells, a recent study reported on the ability of DON and its metabolite 3-acetyldeoxynivalenol (3ADON) to inhibit the nuclear factor κ B (NF- κ B) signaling pathway induced by tumor necrosis

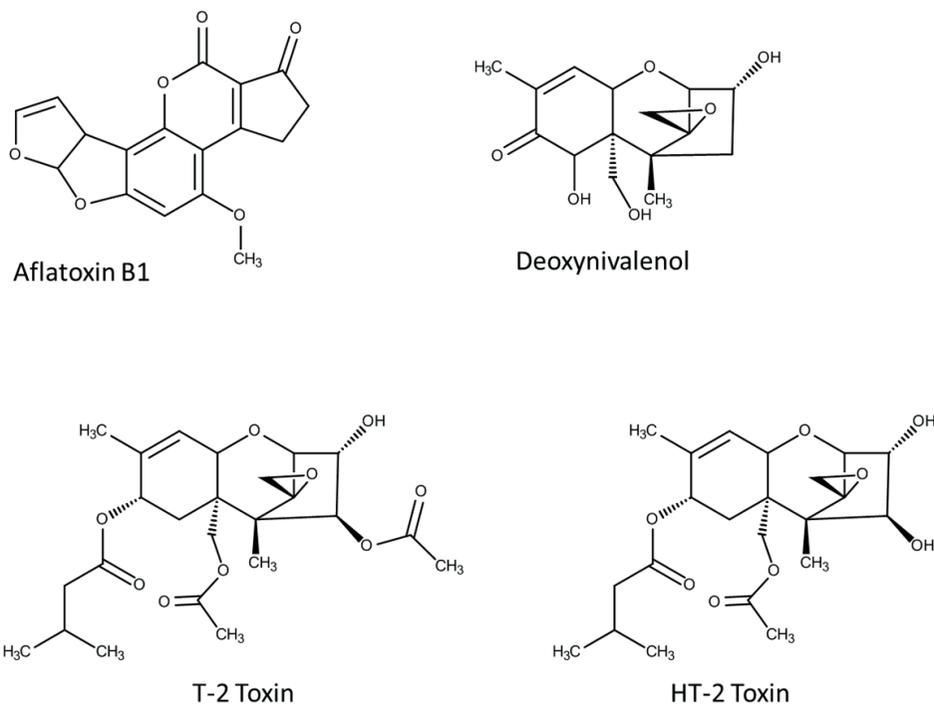


Figure 3: Chemical structure of aflatoxin B1 and trichothecene compounds deoxynivalenol, T-2 toxin and HT-2 toxin.

factor α (TNF- α), leading to the ectodomain shedding of TNF receptor 1 [54].

The ability of DON and T2 to exert endocrine disrupting effects has been studied *in vitro* using human adrenocortical H295R cell lines as a model for steroidogenesis [55]. The authors reported a reduction in hormone levels in media of exposed cells, probably due to a decrease in cell viability at increasing toxin concentrations. In particular, 13 out of 16 steroidogenic genes analyzed by quantitative real time PCR were significantly regulated by DON, T2 and HT2 compared to the control, causing an alteration of the androgen, estrogen, progesterone and glucocorticoid agonist or antagonist responses. Although the knowledge so far does not indicate that DON, T2 and HT2 directly interact with the steroid hormone receptors, this study suggests that DON, T2 and HT2 toxin may potentially act as endocrine disruptors [56].

The possible interaction with nuclear receptors has been recently considered for aflatoxin B1, as well. Besides its cancerogenic activity and genotoxicity, AFB1 has been reported to affect the mRNA expression of several nuclear receptors, such as pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR), in primary cultures of human hepatocytes. Trials were conducted at non-cytotoxic concentrations. These findings preliminarily suggest that AFB1 could activate PXR, CAR, and AhR [57]. In consideration of the severe acute and chronic toxic effects related to AFB1, efforts should be spent in better elucidating the possible interaction with nuclear receptors and the related adverse effects.

Very recently, the possible interaction between AFB1 and vitamin D receptors has been described [58]. According to the authors, the expression of vitamin D receptor (VDR) in osteosarcoma cell line SAOS-2 seemed to be significantly down-regulated by exposure to AFB1. In particular, the authors suggested that the individual polymorphisms in the VDR gene may lead to an increased susceptibility towards AFB1. As a consequence, an increase in the formation of AFB1-DNA adducts as well as a decrease in the expression of the VDR receptor, could be foreseen. If further confirmed, this aflatoxin mode of action may exert an important role in increasing the risk of calcium deficiency and malnutrition-related diseases in Africa [58].

Since AFB1 is indeed often associated to anorexia, very recently Trebac et al. [57] evaluated in rat the impact of this compound on the major hypothalamic neuropeptides regulating feeding behavior, either orexigenic or anorexigenic. As first result, the authors reported on a significant reduction in body weight gain as a result of repeated AFB1 exposure. A dose-related decrease in the expression of all the hypothalamic neuropeptides studied was found in response to AFB1, leading to alteration inducing appetite disorders as macroscopic effect. The same authors investigated also the effect of AFB1 secretogranin II -derived neuropeptide EM66, which is known to affect the control of food intake. Treated animals showed a decrease in the number of EM66-containing neurons in the arcuate nucleus, and a lower expression of secretogranin II.

In the framework of a better understanding of the effects of mycotoxins at relatively low exposure levels, recent

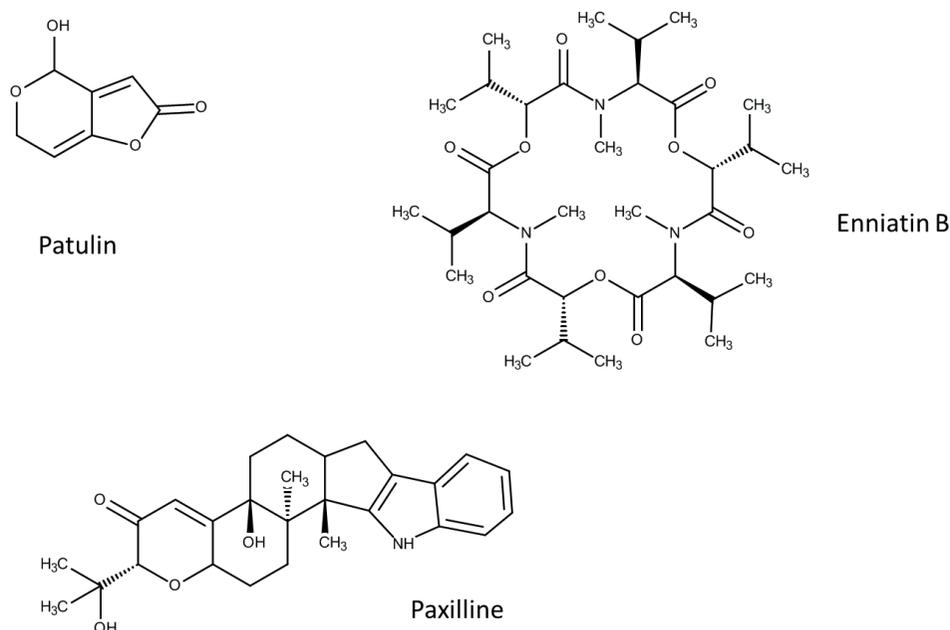


Figure 4: Chemical structure of the emerging mycotoxins patulin, paxilline, and enniatin B.

studies are addressing the possible effects of DON on the neuroendocrine regulation.

Exposure to DON leads to vomiting, food refusal, and anorexia in monogastric mammals. Several recent studies have addressed the mechanisms by which trichothecenes induce these symptoms and revealed a multifaceted action targeting gut, liver and brain and causing dysregulation in neuroendocrine signaling, immune responses, growth [60, 61]

Although studies in the mouse suggest that DON suppresses food intake by aberrantly inducing the release of satiety hormones from enteroendocrine cells found in the gut epithelium, the underlying mechanisms for this effect are not understood. In a recent study, Zhou and Pestka [62] tested the hypothesis that DON-induced hormone exocytosis is mediated by G-protein coupled receptor (GPCR)-mediated Ca²⁺ signaling, using the murine neuroendocrine tumor STC-1 cell line. The results indicate that DON elicits Ca²⁺-dependent secretion of cholecystokinin and glucagon-like peptide-17-36 amide, hormones that regulate food intake and energy homeostasis. Authors showed that these effects were mediated by the GPCR Ca²⁺-sensing receptor and elucidated the underlying mechanism [62].

3.4. Patulin, enniatins, and other minor mycotoxins. Patulin (PAT) is a mycotoxin produced in apples and damaged fruits by various species of fungi, among them the most important is *Penicillium expansum* (see Figure 4). This mycotoxin has been shown to induce toxic effects in animals, a few of which include reproductive toxicity and interference with the endocrine system. With the main aim to deeply understand

its endocrine disrupting potential, Frizzel et al. [63] showed that PAT did not appear to induce any specific agonistic or antagonistic responses in reporter gene assays at the receptor level, although the nuclear transcriptional activity was affected. At higher concentration, a 6 fold increase in the glucocorticoid receptor transcriptional activity was indeed observed in the presence of cortisol. Similarly, at the hormone production level PAT exposure seemed to increase estradiol and progesterone levels, and to decrease testosterone production. The authors, anyway, didn't report any information on the possible interaction of PAT with nuclear receptors leading to these effects.

The possible interaction between mycotoxins and nuclear receptors have been showed for minor compounds as well, leading to a better understanding of the toxic/pharmacologic activities of these metabolites. Among them, paxilline is a tremorgenic alkaloid compound produced by *Penicillium paxillii* (see Figure 4). It is known to be a reversible inhibitor of the cerebellar inositol 1,4,5-trisphosphate (InsP3) receptor. As paxilline does not affect InsP3 binding to the receptor, it can be considered a non-competitive inhibitor [64].

Enniatin B (ENN B) is among the emerging *Fusarium* mycotoxins known to contaminate cereals (see Figure 4). Its potential activity as endocrine disruptor has been recently reported human adrenocortical H295R cell lines and neonatal porcine Leydig cell models by Kalayou et al. [56].

At the receptor level, ENN B did not appear to induce any specific agonistic or antagonistic responses in reporter gene assays, although cell viability was significantly affected at the highest concentration. Measurement of hormone levels in H295R cells revealed that the production of progesterone,

testosterone and cortisol in exposed cells were reduced, but the level of estradiol was not significantly affected. Gene transcription analysis in H295R cells showed that twelve of the sixteen genes were significantly modulated by ENN B compared to the control already at low concentration. These results suggest that adrenal endocrine toxicity is an important potential hazard for ENN B exposure.

4. Conclusion

An estimated 500 million of the poorest people in sub-Saharan Africa, Latin America, and Asia are exposed to mycotoxins at levels that substantially increase mortality and severe diseases [65–67]. Significant negative effects of aflatoxin on child growth and immune modulation have been reported. This evidence indicate that, in spite of intensive research over decades, there is still a lot to understand about the mycotoxin effects and mode of action.

Studies addressing the issue at molecular and mechanistic levels are thus strongly required – mainly in consideration of possible endocrine or neuroendocrine disruption activity. Many mycotoxins, especially those from *Fusarium* spp, are responsible of immunosuppressive activity and inflammatory pathway dysregulation, although little is known about the mode of action at molecular level. Since nuclear receptors, and among them LXR and PPAR, are involved in the regulation of the inflammatory pathway, their possible involvement in the immunosuppressive activity of mycotoxins is worth of investigation. In addition, it must be considered that a large number of mycotoxins occur in crops simultaneously. Therefore, the additional, synergistic, or agonistic effects of these compounds should be addressed. Similarly, the co-exposure to other natural toxicants or endocrine disruptors have to be carefully managed, especially when toxicological effects affecting children growth are taken into account.

In this framework, the use of innovative methodologies from different scientific fields may support the future research. In particular, the use of reliable methods for predictive toxicology as well as of in silico techniques from computational chemistry will speed up the research by identifying possible and multiple targets. Multi-target in vitro methods should be developed, to allow the elucidation of different endpoints and the evaluation of additive/synergistic/agonistic effects. Finally, the possible interaction of natural toxicants and bioactive compounds naturally occurring in food should be taken into consideration as well, to return a full picture of the xenobiotics entering and affecting the living organism.

Competing Interests

The author declares that there is no competing interests.

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