A Brief Overview of our Current Understanding of Nivalenol: A Growing Potential Danger yet to be Fully Investigated

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ABSTRACT

Mycotoxins are secondary metabolites that are secreted by fungi into their direct environment. Recent epidemiologic surveys indicate a significant increase in the global presence of fungal species. Nivalenol, a mycotoxin belonging to trichothecenes type B, has been recently shown to possess one of the more potent toxicities among mycotoxins of this group. It is critical that further research on the toxicological potential of nivalenol on humans and animals be undertaken. This review reports on the studies conducted on nivalenol thus far and in so doing serves to provide a basis for our current understanding of the mycotoxin.

Keywords: Mycotoxins; Nivalenol; Toxicity; Health Concern; Tricothecenes.

INTRODUCTION

Mycotoxins are secondary metabolites that are produced by fungi into their direct environment. Recent epidemiologic surveys indicate a significant increase in the global presence of fungal species, probably as a consequence of global climate change (1). Favorable temperature and moisture levels are crucial for mycotoxicigenic fungi and mycotoxin production. In general, tropical climates favor aflatoxin production while ochratoxin A, patulin and *Fusarium* toxins (e.g. deoxynivalenol, nivalenol) are more of a concern in colder regions (2). Mycotoxins are currently the most frequently detected contaminants in animal feeds and certain plant-derived foods (3). Mycotoxins originate from different biosynthetic pathways, resulting in a large diversity of chemical structures and biological effects (4). Fungal invasion and propagation in living plants (pre-harvest contamination) is influenced by various signaling molecules, and plant stress factors stimulate mycotoxin production (3). Despite numerous efforts in the areas of plant genetics and plant protection, the persistence of fungal species in the environment and their complex life cycle makes it impossible to entirely avoid fungal invasion and mycotoxin contamination of crops at the pre-harvest stage under the current conditions of agricultural practice (5).

The actual ecological role of mycotoxins is still incompletely understood. Secondary metabolites, particularly those of endophytic fungal species, exert antibiotic, anthelmintic and insect repelling properties and may protect plant seeds and plant tissues from external damage (1). At the same time, the mycotoxins of many fungal species have a harmful effect, competing with plants for nutrient resources and damaging plant tissues sometimes leading to plant disease (e.g. *Fusarium* head blight or FHB) (6). Plants react to fungal invasion using protective mechanisms, for example, some plants can modify mycotoxins via conjugation reactions, predominantly by glucoside binding (7). These modifications result in new molecules with different physicochemical structures (modified mycotoxins) that have remained previously undetected (masked mycotoxins, including *Fusarium* mycotoxins which are not usually detected by routine testing) (8).

Trichothecenes are a large group of mycotoxins mainly

produced by fungi of the *Fusarium* genus (9). Worldwide, these toxins are commonly found in cereals, particularly wheat, oats, barley and maize (10, 11). Cereals are commonly used in feed, and farm animals may therefore consume relatively high amounts of trichothecenes. Trichothecenes are toxic to animals and exposure has been linked to reproductive disorders in domestic animals (12). Because of their effects on the immune system, exposure to trichothecenes could also facilitate the development of infectious diseases in humans and animals (13). Trichothecenes are closely-related sesquiterpenoids (which all possess a ring structure) with a 12, 13 epoxy ring and a variable number of hydroxyl, acetoxy or other substituents. Trichothecenes have been classified into types A, B, C and D, based on their functional group (14). Type A and type B trichothecenes have a wide range of toxic effects on production animals and humans (15). Type A trichothecenes are represented by T-2 toxin, HT-2 toxin and diacetoxyscirpenol (DAS). Type B trichothecenes are most frequently represented by deoxynivalenol (DON), nivalenol (NIV) and fusarenon X (FX) (9) (Fig.1; Table A). C and D types are not covered in this review as type C has not been associated with adverse effects in livestock. In contrast to type C trichothecenes, type D trichothecenes are potent cytotoxic compounds however no naturally occurring toxicoses have consistently been attributed to these mycotoxins (16).

NIV (12, 13-epoxy-3,4,7,15-tetrahydroxytrichothec-9-en-8-one) belongs to trichothecene type B, produced by *Fusarium graminearum*, *F. crookwellense* and *F. nivale* (17, 18). These fungi can inhabit various cereal crops (wheat, maize, barley, oats and rye) and grain based food products (bread, malt and beer) (11). The *Fusarium* species invade and grow on crops, and may produce NIV under moist and cool

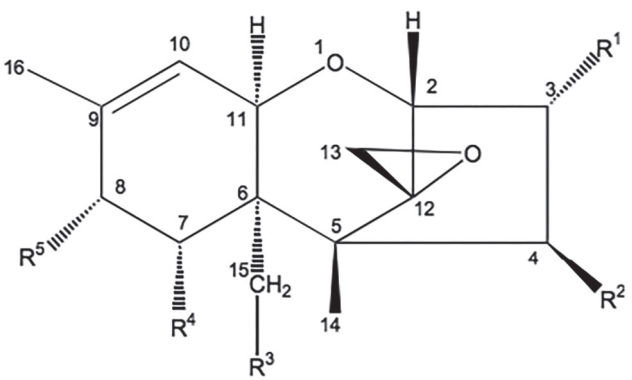

Figure 1: Chemical structure of trichothecenes type A and type B. Substitutions R1 through R5 are given above.

conditions. In recent reports, NIV has been detected in cereal based products in European countries (19, 20, 21), Brazil (22), Japan (23), Southeast Asia (24) and China (25). The average concentration of NIV contamination is dependent on the geographical area where the contamination occurred (from 20–60 μg/kg in France, to 584–1780 μg/kg in China) however no country has been found to be without some level of contamination (26). The European Food Safety Authority (EFSA) has issued guidelines on the risk to human and animal health related to the presence of NIV in food and feed in the form of a tolerable daily intake (TDI) of 1.2 μg/kg bw/day (27). In contrast, the Food Safety Commission in Japan (FSCJ) has established a TDI of 0.4 μg/kg bw/day (28).

NIV is thought to act by binding to the ribosomal peptidyl transferase site to inhibit protein and DNA synthesis (29). Consequently, exposure results in decreased cell proliferation (29) and apoptosis, particularly in organs containing actively dividing cells such as the small intestine, thymus, spleen, bone marrow and testes. It also causes decreased cell proliferation and apoptosis in mitogen-stimulated human lymphocytes, as observed with other trichothecenes (30, 31, 32, 33, 34). Several recent reports have described additive/synergistic toxicity due to a combination of trichothecenes, these situations have resulted due to the natural and common co-occurrence of several different trichothecenes in crops (e.g. NIV and DON) (4, 35). These same studies also detected a greater toxicity of NIV on epithelial cells compared to other well-known trichothecenes (36). Despite its relatively potent toxicity, NIV has been poorly investigated in comparison to other mycotoxins belonging to the same family.

To date, the data on toxicity of NIV are incomplete. Limited data are available and its toxicokinetic profile has only been reported in pigs and mice (37, 38). The aim of this review is to collate the relevant literature about NIV. Given the growing rate of NIV contamination and the likely serious consequences of this, it is vital that we rapidly generate data to increase our understanding of this mycotoxin. Hence, the aim of this review is to stimulate scientists to perform research in this field and in addition, to help authorities gather data for risk assessment in humans and animals.

METABOLISM

Once present in animal tissues, trichothecenes undergo a variety of different metabolic reactions including hydrolysis to split off side groups, hydroxylation and de-epoxidation.

Table A: The chemical structures of the substituents R1-R5 are type A and type B trichothecenes (37)

Trichothecene	R1	R2	R3	R4	R5
			Type A		
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
Diacetoxyscirpentriol	OH	OAc	OAc	H	H
			Type B		
Deoxynivalenol	OH	H	OH	OH	O
Nivalenol	OH	OH	OH	OH	O
Fusarenon X	OH	OAc	OH	OH	O

NIV has been reported to be metabolized to a de-epoxidated form by microorganisms in the gastrointestinal tract (39). The intestinal microflora is important for the biotransformation of trichothecenes. The presence or absence of particular intestinal microflora species can influence the extent to which an animal is sensitive to NIV as it has been demonstrated that the de-epoxidated products are less toxic than the parental molecules (40). The removal of the epoxide oxygen at the carbon 12 and 13 position has been reported to be a significant single step detoxification reaction for trichothecenes (Fig.2). Indeed, the rate of de-epoxidation before absorption is an important factor in the toxicity of NIV and other trichothecenes. The highest toxicity is expected in animals with no de-epoxidation. For example, the deepoxy T-2 toxin (DE T-2) metabolite of T-2 toxin demonstrated a toxicity that was reduced 400 fold when compared to that of the parental compound in the rat skin irritation bioassay (40). The de-epoxides of NIV and DON were shown to be 51 and 24 times less cytotoxic than the corresponding toxins with an intact epoxide ring (41).

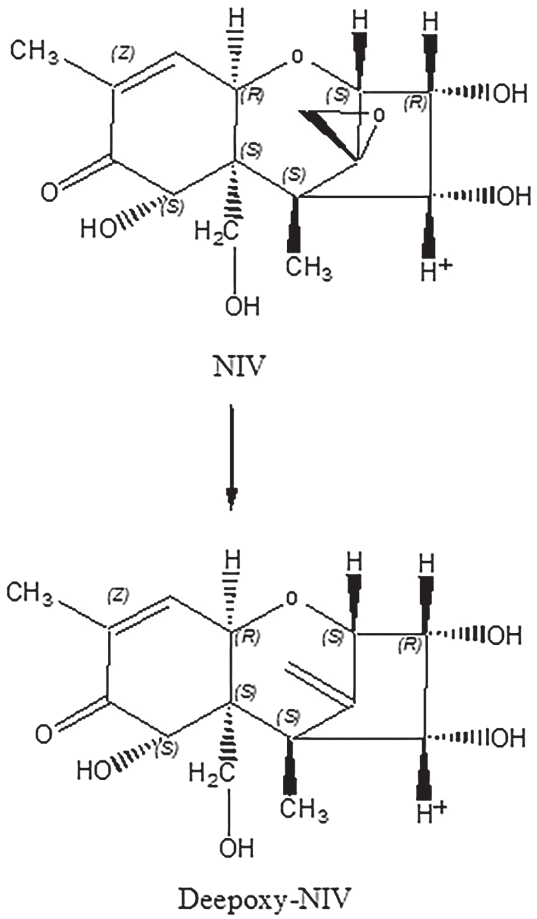

Figure 2: Detoxification path way of NIV into deepoxy-NIV in animals.

Rodents

The first study concerning *in vivo* metabolism of NIV was reported in rats (42). Male Wistar rats (n=5) were orally administered with NIV (5 mg/kg bw) 12 times at 2- or 3-day intervals. Either urine or feces were collected daily for 39 days. The results showed a large amount of an unknown metabolite in the feces. The unknown metabolite was then identified as 3,4,7,15- tetrahydroxytrichothec-9, 12-dien-8-one, namely de-epoxy NIV. Poapolathep *et al.* (38) studied the fate of NIV and FX in mice using ^{3}H-NIV and ^{3}H-FX. Radioactivity was measured mainly in the urine in mice given ^{3}H-FX, and mainly in the feces in mice given ^{3}H-NIV. It was demonstrated that the radioactivity was mainly due to NIV and in a small part, to an unknown metabolite (which was never structurally identified). In addition, it was shown that FX-to-NIV conversion mainly occurred in the liver and kidney. Another study showed that NIV has potential to be transferred in unchanged form to fetal or suckling mice via the placenta and milk, respectively. It has been demonstrated that the fraction of NIV in maternal milk is mainly due to the

amount of FX previously converted to NIV in the maternal body (35). According to these findings, mice (lacking the de-epoxy-NIV) might be more sensitive to NIV toxicity compared to rats.

Swine

In pigs fed twice a day for a week with a diet containing NIV (0.05 mg/kg bw), NIV was mainly excreted in feces. No metabolites of NIV, either as glucuronic acid or sulphate conjugates, or as de-epoxy-NIV, were found in plasma, urine, and feces, indicating a lack of metabolism. It has been speculated that the inability of their intestinal microbes to de-epoxidate trichothecenes might increase the sensitivity of pigs to trichothecene toxicity. The high NIV concentrations in feces (higher than in the feed) suggested a likely risk of intestinal damage when microbial detoxification ability is lacking (37). In order to investigate if the ability to form de-epoxy-NIV was time – dependent, pigs were fed with higher doses of NIV (2.5 or 5 mg/kg bw) for 3 weeks. The results showed that all pigs were able to metabolize NIV to de-epoxy-NIV after one week of treatment (not before) and de-epoxy-NIV concentration was highest at the end of the NIV treatment. From these results, it was concluded that pigs are able to convert NIV into de-epoxy-NIV only if exposed to this toxin for a prolong period (37).

Ruminants

Trichothecenes are, to a large extent, de-epoxidised in the rumen of the cattle before absorption into the blood, this is regarded as a detoxification process (43, 44). Data available for NIV in ruminants are scarce. To the best of the authors' knowledge, only one study is reported. This study involved the incubation of NIV with cow ruminal fluid and found that a large fraction of NIV (78-82%) was transformed to deepoxy-NIV (37).

Poultry

Broiler chickens were fed with NIV (2.5 or 5 mg/kg) for 3 weeks (37). De-epoxy-NIV was not detected in any samples of feces. The unidentified metabolite of NIV was found in the feces samples, this was nonspecifically identified as an acetylated metabolite of NIV. A subsequent *in vitro* study monitored the degradation of 12 trichothecenes (including NIV) by chicken intestinal microbes (45). NIV was found to be converted to de-epoxy-metabolite by the chicken large intestinal microbes. These studies are conflicting, and different processes of the intestinal microbes *in vivo* versus *in vitro* might have triggered this difference. On the other hand, NIV and de-epoxy-NIV were found in feces of laying hens at levels up to 10% of the ingested NIV (5 mg NIV/kg diet for 50 days) (46).

TOXICITY

NIV and other trichothecenes are potent inhibitors of protein synthesis and their toxicity is directed at RNA and DNA as well as mitochondrial and electron transport chain functions (9). In addition, NIV can stimulate lipid peroxidation, alter cell membrane function and modulate immune responses. It activates mitogen activated protein kinases (MAPKs) through the ribosomal stress response, this could be another mechanism of toxicity that operates via apoptotic and pro-inflammatory processes (9). Rapidly dividing cells, such as intestinal epithelial cells or immune cells, are especially sensitive to NIV and other type B trichothecenes. The 50% lethal dose (LD_{50}) values, which may differ based on route of exposure and species exposed, can be used to compare the toxicity of trichothecenes and are shown in table 1. Various studies have commonly detected NIV, often together with DON in cereals worldwide (20, 24, 44, 47). Furthermore, the toxicity of NIV is higher than that of DON as showed from the LD_{50} in mice (intra-peritoneal administration) of 4 mg/kg versus 70 mg/kg, respectively (Table 1). Incidentally, human health authorities tend to assume that NIV toxicity is similar to DON toxicity (27), since the chemical structures of these two toxins are similar. NIV and DON also share toxicological properties, such as the inhibition of cell proliferation, induction of interleukin-8 secretion, and the involvement of stress-activated MAPKs and nuclear factor κB in the signal transduction pathways of their toxicities (48). Therefore, NIV should be of concern for food safety but *in vivo* information for assessing the health risk remains scarce.

The intestinal epithelial cell is a recognized target for NIV and damage is likely to result in impaired absorption of nutrients (sugar and electrolyte) leading to the known detrimental effect of trichothecenes on animal growth (49). The exposure of intestinal epithelial cell to NIV may alter their ability to proliferate and to ensure a proper barrier function. Several reports have shown that NIV has a greater toxic impact on the gastrointestinal tract than DON. Cheat *et al.* (50) reported that *in vivo*, proliferative cells of pig intestinal

Tabel 1: Partial list of type A and B trichothecene toxins and their comparative toxicity (adapted from Haschek *et al.* (26))

Group	Main producer	Trichothecenes	Acute LD_{50} values (mg/kg)				
			Mouse i.v. or i.p.[a]	Mouse oral or p.o[a]	Mice p.o	Fisher 344[b] Rat p.o	Fisher 344 Rat subcutaneous[c]
A	*Fusarium spp.*	T-2 toxin	3.0-5.3	3.8-10.5			
		HT-2 toxin	6.5-9.0				
		Diacetoxyscirpenol (DAS)	9.6-23.0	15.5-46.0			
B	*Fusarium spp.*	Deoxynivalenol (DON, vomitoxin)	70.0-76.7	46			
		Nivalenol (NIV)	4.0-6.4		38.9	19.5	0.9
		Fusarenon X (FX)	3.4	4.5			

[a] Summarized by Haschek and Beasley, (26)
[b] Kawasaki *et al.* (32)
[c] IARC. (29)

mucosa showed a 30% and 15% decrease (in number) after NIV and DON exposure, respectively. Other recent studies also concluded that NIV induced a strong dose-dependent cytotoxicity on intestinal epithelial cells *in vitro* (36, 51, 52). A normal porcine jejunal epithelial cell line (IPEC-J2) was exposed to various *Fusarium* mycotoxins and resulting cell viability was observed: The results showed that the toxicological potency rank was NIV > DON > ZEA > FB_1 and all combinations of mycotoxins gave reduced cell viability (52).

Apoptosis is now recognized as a major mechanism for trichothecene-induced toxicity (9). The induction of apoptosis by trichothecenes, especially T-2 toxin, FX and NIV have been studied in animals (31, 38, 53, 54). The early morphologic changes in immune cells, such as those within lymphoid tissues (thymus, spleen and Peyer's patches) were described as apoptosis caused by NIV. It was shown that, when NIV was given orally to mice at the dose levels of 5, 10, 15 mg/kg bw, the degree of apoptosis was dose-dependent (33). In another study, NIV was incubated with human blood cells (human K562 erythroleukemia cell line). The results showed that NIV was toxic for human blood cells causing DNA damage and apoptosis (55). NIV also produced polyribosomal degradation in bone marrow cells and significant erythropenia and slight leukopenia in mice treated with 30 mg/kg of NIV (29). Both the *in vivo* and *in vitro* findings indicated that NIV induces apoptosis in different immune cells, probably decreasing their functional properties. Therefore the immune system seems to be one of the targets of NIV. However, there is no experimental evidence on mutagenic and/or carcinogenic properties of NIV in animals as specified by International Agency Research on Cancer (IARC) (56). NIV was classified as group 3, indicating that the toxin is not considered carcinogenic to humans (56).

Apart from these findings, NIV has reported effects on the reproductive and developmental system. In a study by Ito *et al.* (57), pure NIV was injected intra-peritoneally in pregnant albino Switzerland (ICR) mice at dose levels of 0, 0.1, 0.5 or 1.5 mg/kg bw/day on days 7-15 of gestation. The highest dose caused stillbirths after vaginal hemorrhage in 6 out of 10 animals. High embryo lethality was recorded in the two highest dose groups (88 and 48%). No fetal malformations were observed in the treated groups. A single administration of 3 mg/kg bw on day 7 affected the embryo within 10 hours, damaged the placenta within 24 hours, and caused abortions at 48 hours.

The general toxicity and immunotoxicity/hematotoxicity of NIV are considered to be significant. As discussed previously, these effects are similar to those of other trichothecenes. NIV has been shown to be more toxic than the other type B trichothecene (DON) even though it is a less common crop contaminant than DON (23).

It should be noted that the risk for animals and public health relates to the presence of NIV in food. NIV-induced health damage is regarded as a serious problem and NIV is considered to be one of the mycotoxins that need to be regulated.

In conclusion, animal exposure to NIV is primarily from consuming cereal grains and cereal by-products. NIV can also be a potentially dangerous mycotoxin for humans. The available information on the toxicity of NIV is incomplete

and it is critical this shortfall is remedied as soon as possible. NIV is of significant and growing concern and it is the responsibility of the scientific community to propagate specific information on its toxicity. It should be remembered however, that the major challenge in mycotoxin risk assessment is understanding the effects of the total mycotoxin exposure, including being able to predict interactions between different mycotoxins at the level of adsorption and effects on target organs. While initially risk assessment focused on avoidance of probable carcinogenic mycotoxins, animal health concerns are now related to undesirable effects on intestinal health and the immune system, reproductive performance and sensitivity to vaccination and to the mitigation of adverse effects in farm and companion animals.

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