Nivalenol (Fungal Sesquiterpenes)

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Contents

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Abstract

Nivalenol (NIV) belongs to the B-type trichothecene mycotoxins produced by *Fusarium* species. The occurrence of NIV contamination is limited to certain areas around the world, such as Japan, Korea, New Zealand, and a part of Europe, where it has had adverse effects on human and animal health. This chapter focuses on the mycology, occurrence, biosynthesis, toxicology, methods of analysis, and risk assessment of NIV.

Keywords

Analysis method • biosynthesis • exposure assessment • mycology • nivalenol • toxicity

Abbreviations

bw	Body weight
DON	Deoxynivalenol
ECEU	European Commission Union
EFSA	European Food Safety Authority
FHB	Head blight
FX	Fusarenon-X (4-acetyl NIV)
JECFA	Joint expert committee of food additives
$LD_{50:}5$	50% lethal dose
LOAEL	Low-observed-adverse-effect level
LPS	Lipopolysaccharide
NIV	Nivalenol
S9	S9 liver microsomal enzymes
SCF	Scientific Committee on Food
ZEN	Zearalenone

1 Introduction

Nivalenol (NIV) was discovered by Japanese scientists from the culture medium of *Fusarium nivale* strain Fn-2B obtained from *Fusarium* head blight-infected wheat, in Japan [1]. Subsequently, Fn-2B was reclassified as a new species, *F. kyushuense*

O'Donnell & T. Aoki [2] from molecular phylogenetic analyses. Other Japanese researchers identified the chemical structure of NIV, fusarenon-X (4-acetyl NIV), and deoxynivalenol (DON) [3–6].

Trichothecene mycotoxins are the main mycotoxins produced by *Fusarium* species. Although there are macrocyclic and non-macrocyclic mycotoxins, the latter primarily contaminate wheat, barley, and maize and are classified into two types: Type A, including T2 toxin and HT2 toxin, diacetoxyscirpenol, and neosolaniol and type B, including DON, NIV, and 4-acetyl NIV. T2 toxin is reportedly the causative agent of outbreaks of foodborne diseases that occurred in the Orenburg region of the USSR during the 1930s–1940s. This outbreak was termed alimentary toxic aleukia (ATA), and symptoms of this disease include nausea, emesis, diarrhea, leukopenia, hemorrhages, and shock-mediated death.

Although the outbreak caused by type B trichothecenes is less severe than ATA, acute human illnesses caused by the consumption of *Fusarium*-infected wheat and barley have been reported in Japan, India, and China [7–9]. In these outbreaks, DON, NIV, and zearalenone were commonly detected in food.

Concerning type B trichothecenes, chronic and acute adverse health effects are of considerable concern. DON and NIV have been reported to suppress the immune system, depending on the dose and frequency of exposure [10].

Compared to DON, the distribution of NIV-producing fungi is limited and lacks toxicological and exposure data. DON has been evaluated by the FAO/WHO Joint Expert Committee of Food Additives while NIV has not. In countries where NIV contaminates cereals, the adverse health effect induced by NIV is a serious problem, and NIV is considered to be one of the mycotoxins whose risk needs to be assessed and regulated. In Europe, a large-scale surveillance of trichothecenes in food from 2000 to 2002 revealed the occurrence of NIV contamination [11]. In Japan, as NIV-producing fungi also occur, the occurrence of DON and NIV were surveyed in food. Risk assessment has been completed by the Food Safety Commission (FSC) of Japan. The European Commission's (EC) (now the European Union, or EU) Scientific Committee on Food (SCF) evaluated and determined a provisional daily tolerable intake as 0.7 μg/kg of body weight (bw) [12], but the FSC of Japan concluded that this level should be 0.4 μg/kg of bw.

This chapter reviews the latest findings on the mycology, biosynthesis, occurrence, toxicology, analytical methods of analysis, and risk assessment of NIV.

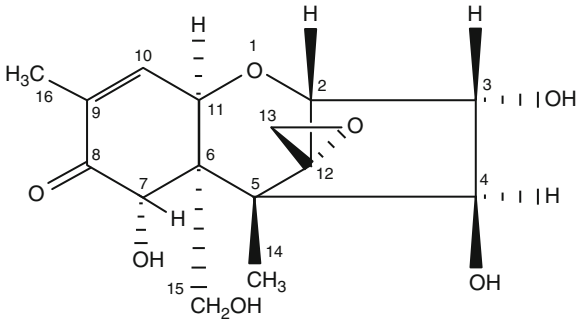
2 General Biology

2.1 Common/Systematic Name

CAS (No.23282-20-4)
3α, 4β, 7α, 15-tetrahydroxy-12, 13-epoxytrichothec-9-en-8-one, IUPAC
12, 13-epoxy-3α, 4β, 7α, 15-tetrahydroxytrichothec-9-en-8-one

2.2 Molecular Formula/Molecular Weight

$C_{15}H_{20}O_7$: 312. 32



Nivalenol

2.3 General Characteristics

Crystals from methanol; mp., 80–90 °C; dried in presence of P_2O_5 in reduced pressure; mp., 222–223 °C, tetraacetate; mp., 168–170 °C, $[\alpha]_D^{24}$+ 21.54° (c = 1.3, in EtOH)

2.4 Spectral Data

λ↓Max ↑MeOH 218 Nm (Ɛ = 7,500), Tetraacetate, 227 Nm (Ɛ = 7,900)

3 Mycology

Fusarium head blight (FHB) is a harmful disease that infects wheat, barley, and other cereals. This disease not only reduces grain yield and quality but also causes the contamination of trichothecene mycotoxins such as DON and NIV and other mycotoxins in the grain. The pathogens of FHB, *Fusarium* species, can be classified into two chemotaxonomic groups, the DON chemotype and the NIV chemotype [13–15]. Lee et al. [14] clarified that a single gene (*Tri13*) is responsible for the differential ability to produce DON or NIV. *Fusarium* DON chemotypes are found worldwide, while NIV chemotypes are found in more restricted regions, namely, limited areas of Asia, Africa, Europe, and North America [16, 17]. *F. kyushuense* and *F. graminearum* complex species (*F. asiaticum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. poae*) are also reported to produce NIV (Table 100.1).

The *F. graminearum* species complex [*F. graminearum* Schwabe; teleomorph: *Gibberella zeae* (Schwein.) Petch] is a species complex consisting of at least nine

Table 100.1 NIV-producing fungi

Fusarium species	Production of mycotoxins		Commodity	Country/region
	DON	NIV		
F. graminearum complex species	+	+	Wheat variety, rice, maize	Whole world
F. graminearum	+	-	Wheat variety, rice, maize	Temperate zone (cold zone of the northern hemisphere, especially): Japan (all), Korea, China
F. asiaticum	-	+	Wheat variety, rice	Temperate zone (especially warm temperature regions): Japan (Honshu and the south), Korea, China
F. vorosii	+	-	Wheat	Japan (Hokkaido), Hungary
F. culmorum	+	+	Wheat variety, maize	Temperate zone (especially cold regions): Europe, Asia, Africa, North and South America, Oceania
F. crookwellense	-	+	Wheat variety, maize	Temperate zone (especially cold region): Japan (Hokkaido)
F. equiseti	-	+	Wheat variety, maize	Subtropics, temperate zone
F. kyushuense	-	+	Wheat variety, rice	Japan (western Japan), China
F. poae	-	+	Wheat variety, maize	Temperate zone (especially cold regions): Japan (Hokkaido)
F. pseudograminearum	+	-	Wheat variety	Chiefly Australia

biogeographically structured lineages based on molecular phylogenetic analyses using worldwide collections. The lineages are as follows: lineage 1 is *F. austroamericanum*, lineage 2 is *F. meridionale*, lineage 3 is *F. boothii*, lineage 4 is *F. mesoamericanum*, lineage 5 is *F. acacia-mearnsii*, lineage 6 is *F. asiaticum*, lineage 7 is *F. graminearum* s. str., lineage 8 is *F. cortaderia*, while lineage 9 is *F. brasilicum*. On potato dextran agar (PDA), the *F. graminearum* species complex produces abundant white mycelia that become yellow to brownish or rose-colored as the cultures age (for 2 weeks); the color of the bottom surface of the colony is usually deep red. The undersurface is usually carmine red (Fig. 100.1). Macroconidia are usually long, slender, and slightly curved to straight, with five to six septa and a well-developed foot cell. Microconidia are absent; chlamydospores are rare but may form in macroconidia [18].

Trichothecene chemotypes of the *F. graminearum* species complex consist of three production groups according to strain differences: DON and 3-acetyl deoxynivalenol (3ADON), DON and 15-acetyl deoxynivalenol (15ADON), and NIV [13–15]. In the *F. graminearum* species complex, lineage classification is not well correlated with the trichothecene chemotype [19, 20].

Regional mycological studies discovered that geographic differences exist among these trichothecene chemotypes [21, 22]. The 3ADON productive group

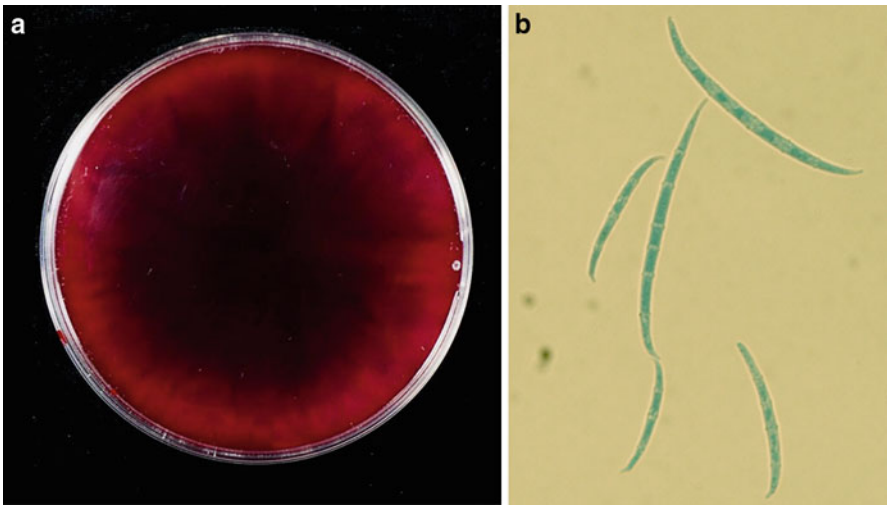


Fig. 100.1 *Fusarium graminearum*. (**a**): giant colony on PDA (*reverse side*) (**b**) Macroconidia

was not detected in 15 strains of *F. graminearum* s. str., and all 13 strains of *F. cortaderia* in New Zealand were of the NIV production group [23]. The NIV production group in *F. asiaticum* has been identified and is likely to represent about 25 % of the population of the *F. graminearum* species complex in Louisiana, USA [24]. In Japan, *F. graminearum* s. str. is predominant in the northernmost island (Hokkaido), while in southern areas, *F. asiaticum* is predominant [25].

In terms of pathogenicity, DON-producing strains are more aggressive and virulent than NIV producers [26, 27]. However, the NIV production group in *F. asiaticum* isolated from the western part of Japan was significantly more virulent than the most virulent DON chemotype *F. graminearum* s. str. strains [28].

Fusarium culmorum (W. G. Smith) Sacc. is the second most important FHB pathogen in wheat and strains of both DON and NIV producers exist. *F. culmorum* inhabits cooler areas such as North, Central, and Western Europe; North America; and Eastern Australia. Strains of both the DON and NIV production groups were isolated from England and Wales: in the south and west of England and Wales, NIV chemotypes are predominant, whereas in the north and east, DON chemotypes are predominant [29].

Fusarium poae (Peck) Wollenw. exists in Europe, North America, and Japan. It is one of the causative fungi causing FHB in small-grain cereals. In the northernmost area of Japan, the NIV production group in *F. poae* plays a role in the contamination of grains with NIV [30]. The NIV production group of *F. poae* was frequently found in Sweden, and NIV contamination has become a considerable concern in Scandinavia [31].

Fusarium crookwellense L.W. Burgess, P. E. Nelson & Toussoun was first isolated in Australia in 1971 as a new species [32]. It is another FHB pathogen

found in small-grain cereals in Poland, New Zealand, China, Canada, Japan, and other countries [33]. *F. crookwellense* isolated from scabby wheat in the northernmost area of Japan produced NIV, 4-acety NIV, and zearalenone (ZEN) when cultured on rice-based medium [30].

4 Biosynthesis

4.1 History of Trichothecene Biosynthesis Studies

In earlier days, biosynthesis studies of trichothecenes were initiated using *Trichothecium roseum* as a model [34]. Having elucidated the scheme of biosynthesis from farnesyl pyrophosphate (FPP; **1**) (see Fig. 100.2), via a cyclized product trichodiene (TDN; **2**), to trichothecin [35, 36], researchers have moved to study *Fusarium* species that produce agriculturally important trichothecenes, such as T-2 toxin, DON, and NIV. Feeding experiments with TDN demonstrated its precursor role in DON biosynthesis [37], as was the case of *T. roseum*. Other postulated intermediates in the biosynthetic pathway were thereafter proven by blocked mutant analysis, precursor feeding experiments, and molecular genetic approaches using *Fusarium* species, including *F. culmorum*, *F. graminearum*, and/or *F. sporotrichioides* [38–40]. Compared to trichothecenes of other fungal genera, *Fusarium* trichothecenes are distinguished by the presence of a hydroxyl or *O*-acetyl at C-3.

4.2 Formation of 12, 13-Epoxytrichothec-9-ene Skeleton (Trichothecene Skeleton): Early Stage of Biosynthesis

In the biosynthesis of *Fusarium* trichothecenes, TDN is oxygenated by a cytochrome P450 monooxygenase (CYP) in the following order: 2α-hydroxylation, 12, 13-epoxidation, 11α-hydroxylation, and 3α-hydroxylation (Fig.100.2). The last oxygenation step proceeds only in *Fusarium* species, which makes *Fusarium* trichothecenes unique among all others (lacking a C-3 substituent) of non-*Fusarium* origin. Under acidic conditions, the resulting fully oxygenated intermediate, isotrichotriol (**3**), appears to undergo second cyclization nonenzymatically to give isotrichodermol (**4**) [41], the first trichothecene intermediate with a toxic 12,13-epoxytrichothec-9-ene skeleton (for a comprehensive review, see ref. [42]). Although isotrichodermol was not isolated as a natural product from wild-type *Fusarium* strains in the biosynthesis studies of many research groups, this may not be unreasonable in view of the importance of 3-*O*-acetylation in trichothecene biosynthesis; perhaps, isotrichodermol must readily be converted to isotrichodermin (ITD; **5**) (see Fig. 100.2) for self-protection of the trichothecene-producing fusaria [43]. ITD, a natural product isolated from a wild-type strain, is further metabolized to various important *Fusarium* trichothecenes [44].

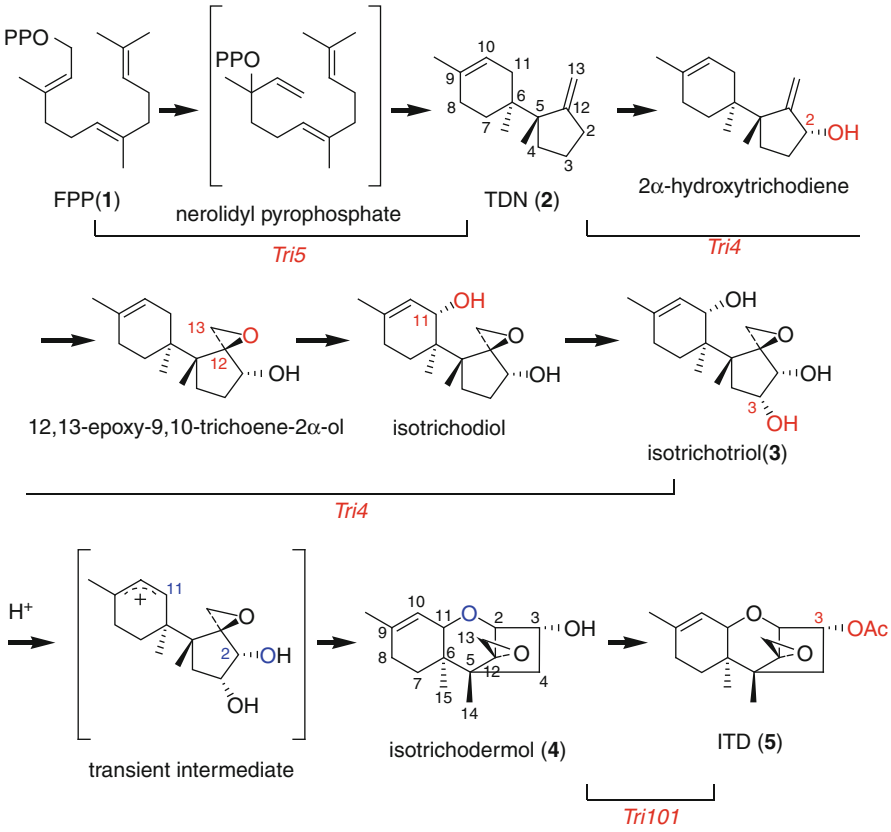


Fig. 100.2 A common pathway to ITD in the biosynthesis of *Fusarium* trichothecenes. These early steps are conserved between type A (e.g., T-2 toxin) and type B (e.g., NIV) trichothecene producers. *Tri* genes that are responsible for these steps are indicated below the half-brackets in red letters. Functional groups highlighted in red indicate that they appeared as the results of expression of these *Tri* genes. Oxygen atom involved in intramolecular attack to C-11 in the nonenzymatic cyclization is shown in *blue*

4.3 Molecular and Genetic Studies of *F. sporotrichioides* as a Basis to Understand NIV Biosynthesis

In 1989, the first isolation and characterization of trichothecene biosynthetic gene was reported for T-2 toxin-producing *F. sporotrichioides* using an antibody raised against a purified enzyme [45]; this gene, designated *Tri5* (formerly *Tox5* in the literature), is responsible for the first committed step in the biosynthesis. Since cosmid clones containing *Tri5* complemented the *tri3*$^{-}$ and *tri4*$^{-}$ blocked mutants, but not the *tri1*$^{-}$ mutant, at least some of the trichothecene biosynthetic genes (*Tri* genes) proved to be clustered around *Tri5* [46]. On the basis of this finding, other *Tri* genes, including *Tri3* and *Tri4*, were identified on this core gene cluster. Three additional *Tri* genes necessary for T-2 toxin biosynthesis, *Tri101* alone and

Tri1 and *Tri16* adjacent to each other, occur separated from the core gene cluster (for reviews, see ref. [42, 47]). Roles of the *Tri* genes in T-2 toxin biosynthesis were examined by molecular genetic approaches, including targeted gene disruption and heterologous gene expression. The functions of *F. sporotrichioides* pathway *Tri* genes (*FsTri* genes) are summarized as follows (see Figs. 100.2 and 100.3):

1. *Tri5* (encoding trichodiene synthase). TRI5 catalyzes cyclization of all-*trans*-FPP via nerolidyl pyrophosphate to TDN [36, 45].
2. *Tri4* (encoding a multifunctional CYP responsible for conversion of TDN to isotrichotriol). *Fusarium* TRI4 [48], which is grouped into a CYP58 family, catalyzes four consecutive oxygenation steps from TDN to isotrichotriol as follows: TDN → 2α-hydroxytrichodiene → 12,13-epoxy-9,10-trichoene-2α-ol → isotrichodiol → isotrichotriol [49].
3. *Tri101* (encoding trichothecene 3-*O*-acetyltransferase). TRI101 catalyzes conversion of isotrichodermol to ITD [50]. Different from other pathway *Tri* genes, *Tri101* was first cloned from *F. graminearum* as a gene that confers resistance to T-2 toxin; subsequent analysis with *F. sporotrichioides* revealed its orthologue in the region of synteny [51]. In addition to isotrichodermol, many trichothecenes, including DON, T-2 toxin, and NIV, serve as good substrates of TRI101 [42]. In the amino acid sequence of TRI101, consensus sequences of acetyltransferases, HXXXDG and DFGWGKP, are found [50]. Apart from its role of self-protection against trichothecenes, C-3 acetyl is essential to serve as substrates of the enzymes in later steps in the biosynthesis.
4. *Tri11* (encoding ITD C-15 hydroxylase). TRI11, the first member of a new CYP family CYP65A1, catalyzes hydroxylation of ITD to give 15-deacetylcalonectrin (15-deCAL; **6**) [52].
5. *Tri3* (encoding 15-deCAL 15-*O*-acetyltransferase). TRI3 catalyzes 15-*O*-acetylation of 15-deCAL to give calonectrin (CAL; **7**) [53]. Other trichothecenes also serve as good substrates of TRI3. However, compared to 3-acetyltrichothecenes, 3-hydroxytrichothecenes are rather poor substrates [54]. The two consensus sequences of acetyltransferases described in the above section (3) were conserved in TRI3 as was the case with TRI101.
6. *FsTri13* (encoding 3-acetyltrichothecene C-4 hydroxylase). In T-2 toxin biosynthesis, FsTRI13 catalyzes hydroxylation at C-4 of CAL [55]. In addition to CAL, other trichothecenes with functional groups at C-8 (and also at C-7) serve as good substrates of FsTRI13.
7. *Tri7* (hypothesized to encode 3-acetyltrichothecene 4-*O*-acetyltransferase). TRI7 is involved in 4-*O*-acetylation of 3, 15-diacetoxyscirpenol (3, 15-DAS; **8**) and its derivatives in T-2 toxin biosynthesis [56]. However, different from TRI101 and TRI3, TRI7 does not possess the consensus sequences conserved among acetyltransferase family; TRI7 shows no amino acid sequence similarity to any other proteins reported so far. Attempts to prepare recombinant TRI7 were not successful, and its enzymatic function has not yet been rigorously proven.
8. *FsTri1* (encoding 3-acetyltrichothecene C-8 hydroxylase). FsTRI1 mainly catalyzes hydroxylation of 3, 4, 15-triacetoxyscirpenol (3, 4, 15-TAS; **9**)

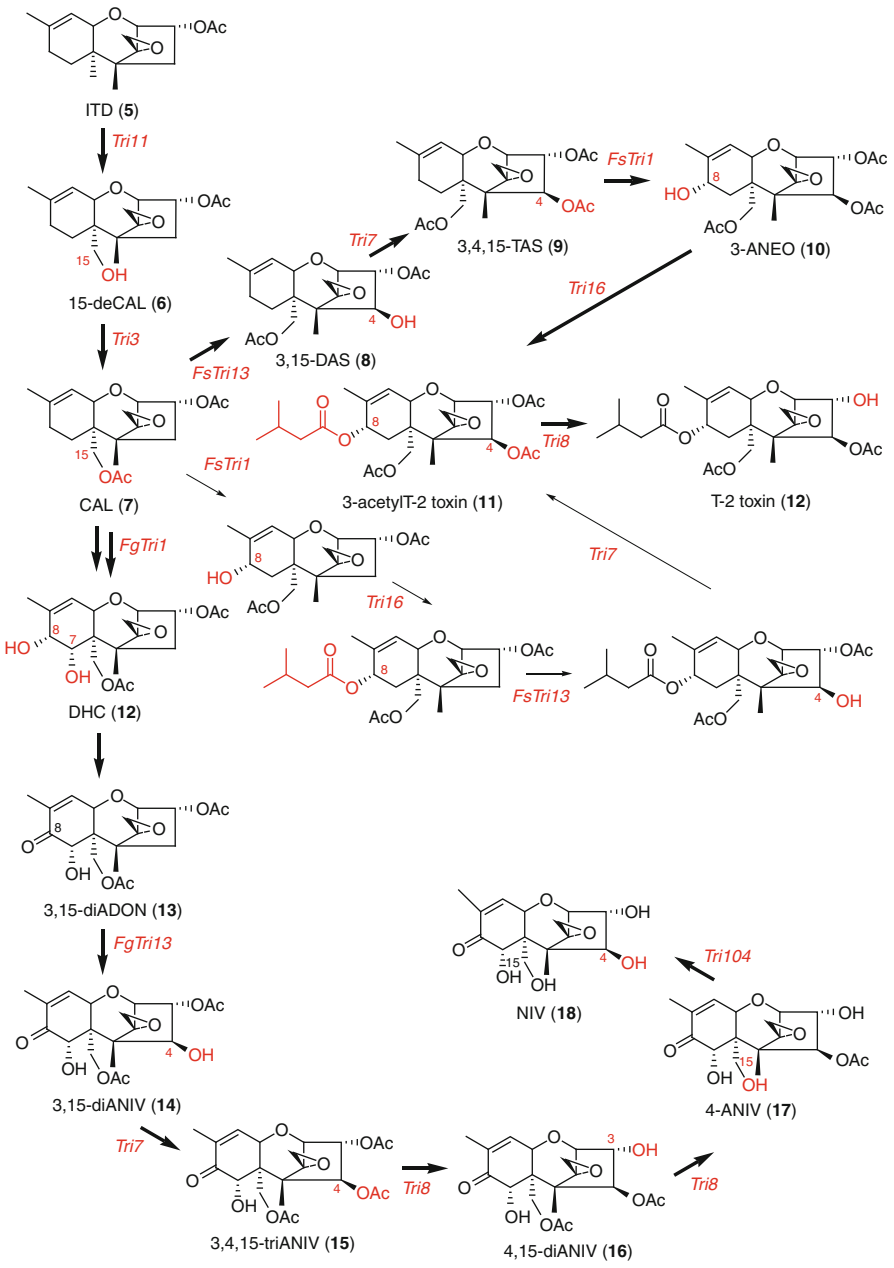


Fig. 100.3 Biosynthetic steps to *F. sporotrichioides* T-2 toxin and *F. graminearum* NIV. Only major pathways to each trichothecene molecule are indicated, and minor pathways are not illustrated. *Tri* genes that are responsible for these steps are indicated in red letters. Functional groups highlighted in *red* indicate that they appeared as the results of expression of these *Tri* genes

(and to a lesser extent CAL) to give 3-acetylneosolaniol (3-ANEO; **10**) (and 8-hydroxycalonectrin) in T-2 toxin biosynthesis [57]. FsTRI1 shows broad substrate specificities and also accept ITD (**5**) and 3, 15-DAS as substrates. The broad substrate specificity of FsTRI1 and FsTRI13 allow branching of the biosynthetic pathway after CAL (**7**) in T-2 toxin biosynthesis (Fig. 100.3).

9. *FsTri16* (encoding an acyltransferase necessary for formation of ester side chain groups at C-8). FsTRI16 mainly catalyzes esterification at C-8 of 3-ANEO to give 3-acetyl T-2 toxin (**11**) [58].
10. *Tri8* (encoding trichothecene deacetylase). TRI8 removes an acetyl from C-8 of the trichothecene skeleton in T-2 toxin biosynthesis [59].

In addition to the pathway *Tri* genes, the core gene cluster contained two regulatory *Tri* genes and one transporter *Tri* gene, which are important for T-2 toxin production: *Tri6* encoding a zinc fingerlike transcription factor [60], *Tri10* encoding a novel protein with a role of other *Tri* gene activation [61, 62], and *FsTri12* encoding a trichothecene efflux pump [63]. *FsTri12* is indispensable for a high level production of T-2 toxin.

The structure of the core gene cluster and the function of the *Tri* genes were conserved between *F. sporotrichioides* and *F. graminearum* [14, 55, 56, 64, 65]. In this way, the identification of *FsTri* genes served as a useful tool for molecular biological analysis of *F. graminearum* that produce type B trichothecenes [66].

4.4 Biosynthesis of NIV

Compared to the structure of T-2 toxin, NIV is characterized by the presence of a keto at C-8 and a hydroxyl at C-7; also, C-4 and C-15 are not acetylated, and instead, hydroxyls are attached to these positions (see Fig. 100.3). To elucidate the biosynthetic pathway of NIV, *FgTri* genes in the core gene cluster were isolated on the basis of nucleotide sequence similarity, and their functions were characterized by the molecular approaches. As to the homologues of *FsTri1* and *FsTri16* that occur outside the core genes cluster, their sequences shared similarity only at the amino acid sequence level; *Tri1* is much more divergent between these *Fusarium* species (viz., 59 % identity between FsTRI1 and FgTRI1), and *Tri16* was found as a pseudogene adjacent to *FgTri1* [67]. The results of targeted gene disruption and heterologous gene expression suggested that most of the enzymes encoded by the *FgTri* genes show the same features as those of *F. sporotrichioides*. However, the following two enzymes exhibit significant differences in their activities depending on their origin:

1. Compared to FsTRI13, the substrates of FgTRI13 appear to be limited to a group of trichothecenes that have a hydroxyl at C-7 and/or a keto at C-8.
2. FgTRI1, but not FsTRI1, catalyzes oxygenation at both C-7 and C-8 of 4-deoxytrichothecenes [68].

Together with the analyses of other *FgTri* genes, a major biosynthetic pathway to NIV is hypothesized to proceed as illustrated in Fig. 100.3: ITD → 15-deCAL → CAL → 7,8-dihydroxycalonectrin (DHC, **12**) → 3,15-diacetyldeoxynivalenol

(3,15-diADON, **13**) → 3,15-diacetylnivalenol (3,15-diANIV, **14**) → 3,4,15-triacetylnivalenol (3,4,15-triANIV, **15**) → 4,15-diacetylnivalenol (4,15-diANIV, **16**) → 4-acetylnivalenol (4-ANIV, **17**) → NIV (**18**). In this scheme, all the biologically acetylatable position, namely, C-3, C-15, and C-4, are once acetylated in this order in the biosynthesis and then deacetylated at a later stage. Recently, a gene responsible for deacetylation at C-4 of 4-ANIV was identified and characterized. This gene, designated *Tri104*, is separated from all other known *Tri* genes in the genome of *F. graminearum* (our unpublished results).

5 Toxicology

5.1 Absorption, Distribution, Metabolites, and Excretion

NIV is mainly absorbed from the intestine, and 11–48 % of administered NIV remains in human bodies until 7.5 h post administration [69]. Acetyl NIV is immediately converted into NIV in serum after intravenous and oral administration. The bioavailability of acetyl NIV was 9.8 % and 19.5 % in broiler chickens and ducks, respectively [70].

In vivo examination using healthy swine showed that NIV was mainly absorbed from the ileum [71]. In vitro examination using a Caco-2 cell line (human intestinal epithelial cell line) showed that apical to basal transportation of NIV was simple diffusion depending on energy [72]. The maximum concentration of ^{3}H-labeled NIV and acetyl NIV was detected in serum after 60 min and 30 min in female ICR mice. The area under the curve of acetyl NIV was 10-fold higher than that of NIV. Acetyl NIV is metabolized in the liver and kidney [72].

NIV is converted by intestinal flora bacteria into less toxic de-epoxy NIV in the intestine as is DON. When NIV was anaerobically cultured with swine feces in vitro, NIV was converted into epoxy NIV. Swine that lacked de-epoxidation ability acquired this ability after their faeces with known de-epoxidation ability (containing as yet unclassified intestinal flora bacteria with de-epoxidation ability) were spread out in pens [73]. When NIV and stomach juice of bovine lumen were anaerobically cocultured in vitro, about 80 % of NIV was converted into de-epoxy NIV [73]. NIV and acetyl NIV are distributed in the serum, liver, kidney, and placenta [74]. Eighty percent of administered NIV was excreted in feces as de-epoxy NIV, while 1 % was excreted in urine as NIV in Wistar rats [75]. In chickens, trace levels of NIV were detected in liver and bile, while NIV and de-epoxy NIV were excreted through the feces in more than 10 % of administered NIV [76]. NIV and acetyl NIV were transmitted to milk in lactating mice [74].

As shown in Fig. 100.4, NIV is metabolized by intestinal flora into de-epoxy NIV and then excreted to urine and feces, while acetyl NIV is converted into NIV in serum and organs.

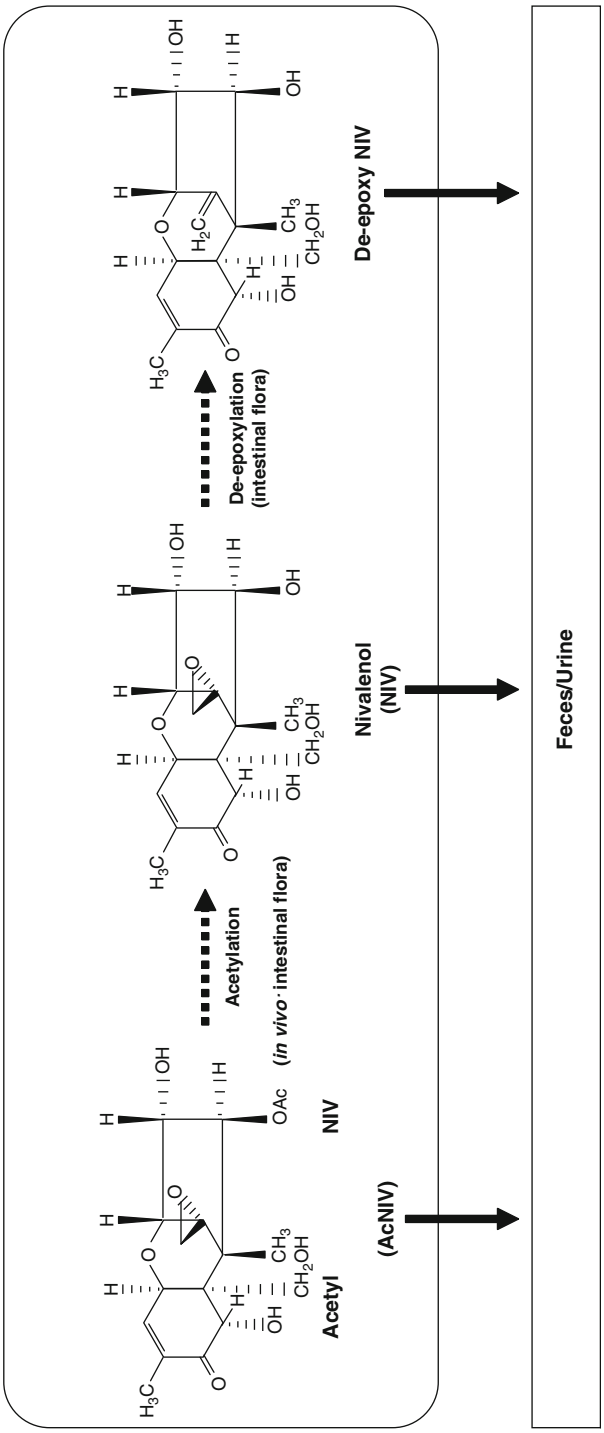


Fig. 100.4 Metabolite pathway of NIV

Table 100.2 LD_{50} of NIV after oral administration

Animal and strains	LD_{50} (mg/kg bw)	References
Mouse, ddY, male, 6 weeks old	38.9	[77]
Rat, F344, male, female, 5 weeks old	19.5	[78]

5.2 Acute Toxicity

Acute toxicity of NIV is shown in Table 100.2.

In 6-week-old male ddY mice, the 50 % lethal dose (LD_{50}) of NIV was 38.9 mg/kg of bw after oral administration, 7.4 mg/kg of bw after intraperitoneal injection, 7.2 mg/kg of bw after subcutaneous injection, and 7.3 mg/kg of bw after intravenous injection. Mice died within 3 days due to the effusion of blood and congestion in the intestine. In 5-week-old F344 rats, the LD_{50} of NIV was 19.5 mg/kg of bw/os and 0.9 mg/kg of bw after subcutaneous injection. Diarrhea and congestion in the gastrointestine were observed. In duck, vomiting was observed after 1.0 mg/kg of bw of NIV, and 0.4 mg/kg of bw of acetyl NIV was administered by subcutaneous injection [79].

5.3 Short-Term Studies of Toxicity

Short-term studies of toxicity were performed in mice, rats, pigs, and chickens, as summarized in Table 100.3.

5.3.1 Mice

C57BL/6 mice were given moldy rice containing at 0, 5, 10, and 30 mg/kg of NIV for 24 days. At 30 mg/kg of NIV, a significant reduction in red blood cell number was observed, but organ weight and the rate of weight gain did not change [80]. When using a diet containing NIV-contaminated rice at 0, 6, 12, and 30 mg/kg for 4 or 12 weeks, weight gain was inhibited in a dose-dependent manner. From these results, LOAEL was estimated to be 0.7 mg/kg of bw [82].

5.3.2 Rats

When Sprague-Dawley rats were treated with diets containing NIV at 0, 6, and 12 mg/kg for 2 or 4 weeks, a significant reduction in feed intake was observed after 1–2 weeks in the group given 0.6 mg/kg of NIV. However, rats recovered after 4 weeks. The low-observed-adverse-effect level (LOAEL) was estimated at 0.6 mg/kg of bw based on the reduction in organ weight [83]. Toxicity studies of single oral administrations using male and female F344 rats showed sedation, eyelid closure, staggering gait, diarrhea, and congestion of the lung and digestive tract. The oral LD_{50} value was estimated at 19.5 mg/kg in both sexes in a repeat oral test, purified NIV was given orally at daily doses of 0.4 and 2.0 mg/kg of bw for 30 days. Takahashi et al. reported subchronic toxicity using F344 rats fed

Table 100.3 Short-term studies of NIV

Study, strain, sex, age, group	Route of administration period	Dose (mg/kg of diet)	Dose (mg/kg bw/day)	Critical effect	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Notes	References
Mouse, C57BL/6, 6 weeks	Diet, contam. 24 days	0, 5, 10, 30	0, 0.6, 1.2, 3.5[a]	Decreasing trend of erythrocytes and leukocytes in a feed of 30 mg/kg Polyribosome damage of bone marrow cells in a feed of 30 mg/kg	3.5[a]	1.2[a]	Used rice mold	[80]
Mouse, C54B16, 7 weeks	Forced oral dosage 3 times per week, 28 days		0, 0.014, 0.071, 0.355, 1.774, 8.870 mg/kg bw, 3 times/week	Increase in plasma phosphate, decrease of urea, alkaline phosphatase activity, and increase of IgG in 8.870 mg/kg bw/day	3.8[b]	0.76[b]		[81]
Mouse, C57BL/6, 7 weeks	Diet, contam. 4 or 12 weeks	0, 6, 12, 30	0, 0.7, 1.4, 3.5[a]	Reduced bw gain and feed intake; dose-dependent increase in serum alkaline phosphatase activity; decrease in adipose tissue	0.7[a]		Used rice mold	[82]
Rat, Sprague-Dawley, 6 weeks	Diet, contam. 14 or 28 days	0, 6, 12	0, 0.6, 1.2[c]	Reduced feed intake (early administration), organ weight changes, increase in CYP2B1/2 of liver microsomal, slight guidance of CYP1A2 in the feed more than 1.5 kg/ml bw	0.6[c]	–	–	[83]
Rat, F344, 5 weeks	Forced oral dosage 30 days		0, 0.4, 2.0	No abnormalities in serum (biochemical and hematological assays) The weight of liver and spleen increased intentionally; however, it did not change after a histopathological inspection using 2.0 mg/kg bw	2.0	0.4	–	[78]
Rat, F344, 6 weeks	Diet, contam. 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	Reduced bw in more than 1.5 kg/ml bw	1.5	0.4	–	[84]
Rat, F344, 6 weeks	Diet, contam. 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	Reduced bw, loose passage, thymic atrophy, decrease in the number of bone marrow cells, diffuse hypertrophy of basophilic cells with	0.4	–	–	[85]

(*continued*)

Table 100.3 (continued)

Study, strain, sex, age, group	Route of administration period	Dose (mg/kg of diet)	Dose (mg/kg bw/day)	Critical effect	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Notes	References
				an increase of castration cells in the anterior pituitary, increase in the atretic follicle in feed with more than 1.5 kg/ml Male bw was reduced by a diet of more than 25 mg/kg Female white blood cell count was reduced by a diet of more than 6.25 mg/kg				
Pig, 51 days (6 males/ group)	Diet, contam. 21 days	0, 2.5, 5		Gastrointestinal erosion and renal disease in some Decrease in spleen cells in the feed of 5 mg/kg Time-dependent increase in the amount of IgA-producing feed more than 2.5 mg/kg	–	–	–	[86]
Chicken, 7 days (6 males/ group)	Diet, contam. 20 days	Experiment I: 0, 0.5, 2.5, 5, Experiment II: 0, 3, 6, 12		Experiment I: Increase in plasma uric acid concentration in the feed containing 5 and 2.5 mg/kg Experiment II: Feed efficiency and rate of weight gain and food consumption were reduced in the feed containing 6 and 12 mg/kg	–	–	–	[87]
Chicken, white leghorn, layer, 55 weeks	Diet, contam. 50 days	0, 1, 3, 5		Decreases in alkali phosphatase, total protein glucose in plasma in feed with 5 mg/kg Gastrointestinal erosion, duodenum internal bleeding, swollen cloaca, and oviducts with immature eggs in feed with 3 and 5 mg/kg Light, enlarged, and fragile livers at 1 mg/kg feed	–	–	–	[76]

[a]Corresponding value of EU Scientific Committee for Food
[b]Value corresponding to dose/day, three times a week
[c]Estimated intake amount by corresponding value of EU Scientific Committee for Food

a diet containing 0, 6.25, 25, or 100 ppm of pure NIV for 90 days. A decrease in bw and loose stools were observed at 100 ppm. In both sexes, bw was also reduced at 25 ppm in males from 6 weeks. In a hematological observation, the white blood cell count decreased after exposure to 100 ppm in males and to 6.25 ppm in females. Based on hematological data, the LOAEL of NIV was determined to be less than 6.25 ppm (corresponding to 0.4 mg/kg of bw/day for both males and females) [86].

5.3.3 Pigs

After pigs were exposed to purified NIV, no feed refusal, vomiting, or change in clinical appearance occurred, but a macroscopic examination showed gastrointestinal erosion and signs of nephropathy at low doses. Exposure to a high dose of NIV decreased the number of spleen cells. Histological data indicated that exposure of pigs to NIV in the diet caused pathological changes in the kidneys and gastrointestinal tract and reduced the number of splenocytes [87].

5.3.4 Chickens

When male broiler chickens were exposed to feed containing NIV, there was an increase in the uric acid concentration in serum, reduction in the rate of weight gain, gastrointestinal erosion, duodenum internal bleeding, swollen cloaca, and oviducts with immature eggs [76].

5.4 Chronic Studies and Carcinogenesis

Two long-term studies have been reported by the same Japanese group. In both studies, moldy rice powder containing NIV was used as the diet. This moldy rice was estimated to contain 3,147 mg/kg of NIV but no fusarenon-X. The first was a 1-year feeding study in which female C57BL/6CrSlc (SPF, 7-week-old mice) were given diets containing 0, 6, 12, and 30 mg/kg NIV; bw gain and feed efficiency showed a dose-dependent correlation. No changes were observed in the liver, thymus, spleen, kidneys, stomach, adrenal glands, pituitary gland, ovaries, sternum, bone marrow, lymph node, brain, and small intestines with or without Peyer's patch portion. Leukopenia was observed in the group administered 30 mg/kg NIV after 6 months and in all NIV-treated groups after 1 year. The LOAEL was determined to be 6 mg/kg of diet (corresponding to 0.68 mg/kg of bw) [77]. The other report was a 2-year feeding study in which the feeding conditions were identical to the 1-year feeding study. A reduction in bw gain was observed in all treated groups of animals. In the group given 30 mg/kg of NIV, leukopenia was observed, but it was not statistically significant. No tumors were found in any of the treated groups. Compared with the ratio of naturally occurring tumors, there was no difference between the treatment group and the control group. The LOAEL was 6 mg/kg feed (corresponding to 0.66 mg/kg of bw) [89] (Table 100.4).

Table 100.4 Long-term studies of toxicity

Study, strain, sex, age, group	Route of administration (solvent), period	Dose (mg/kg of diet)	Dose (mg/kg bw/day)	Critical effect	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Notes	References
Mouse, C57BL/6CrS1c	Diet, contam. 1 year	0, 6, 12, 30	0, 0.68, 1.51, 3.84	Diet group after 6 months at 30 mg/kg, 1 year after NIV in all treatment groups, significant white corpuscle reduction, dose-dependent decrease in absolute weight, and increase in relative weight of liver, kidney, thymus	0.7		Used rice mold	[77]
				Histological abnormality was observed				
Mouse, C57BL/6CrS1c	Diet, contam. 2 years	0, 6, 12, 30	0, 0.66, 1.38, 3.49	Reduced bw gain in all treatment groups	0.7		Used rice mold	[88]
				Decrease in absolute kidney weight in the diet group of 30 and 12 mg/kg				
				Decrease in kidney weight, concentration in the serum of nonesterified fatty acid and alkaline phosphatase increased in the diet group of 12 mg/kg in a dose-dependent manner				
				Tumor could have caused by NIV was not observed				

Table 100.5 Genotoxicity studies of NIV

Endpoint	Test system	Concentrations	Results		References
			Without a metabolic activation system	With a metabolic activation system	
A: In vitro studies					
Sister chromatid exchange	Chinese hamster V79-E cells	5–50 μM/plate	Slightly pos.	Slightly pos.	[89]
Chromosome aberrations	Chinese hamster V79-E cells	5–50 μM/plate	Neg.	Slightly pos.[a]	[89]
Chromosome aberrations	Chinese hamster V79 cells	0.001–0.03 μg/mL	Pos. (3-fold)	N/a	[90]
Chromosome aberrations	Chinese hamster V79 cells	0.03 μg/mL	Pos. (3-fold)	N/a	[91]
Transformation	v-Ha-ras-transfected BALB/3 T3 mouse embryo cells	0.01–0.2 μg/mL	Neg.	N/a	[96]
DNA damage (comet assay)	CHO cells	50–100 μg/mL	Pos.	N/a	[92]
B: In vivo study					
DNA damage (comet assay)	(Male) ICR mice treated with NIV (20 mg/kg bw)		Oral administration: pos. (kidneys, bone marrow, stomach, jejunum, and colon)		[92]
			Intraperitoneal administration: pos. (colon only)		

[a]All aberrations were daughter chromatid exchange
N/a Not tested

5.5 Genotoxicity

Table 100.5 shows a summary of the results of genotoxicity studies of NIV. Some in vitro studies have been reported (Table 100.5A) using V79-E cells (a Chinese hamster lung-derived cell line), NIV induced cell cycle retardation. In the presence of metabolic activation (S9 mix), slight chromosomal aberrations were seen. These effects were nonspecific, suggesting that they were caused by inhibited protein synthesis [89].

In a chromosome aberration test using V79 cells, NIV purified from contaminated corn and barley induced, at 0.001–0.03 μg/mL and 0.03 μg/mL, respectively, a two to three-fold increase in chromosomal aberrations compared with the control [91, 92]. In a short-term transformation assay using v-Ha-Ras-transfected BALB/3 T3 cells, NIV showed no initiation or promotion activity [96]. A single-cell gel electrophoresis

(comet) assay of NIV was conducted using CHO cells and ICR mice (4 males/group). At 50 and 100 μg/mL, NIV damaged the DNA of CHO cells in the absence of a metabolic activation system [92].

In an in vivo comet assay (Table 100.5B), oral treatment with NIV (20 mg/kg bw) resulted in DNA damage in the kidneys, bone marrow, stomach, jejunum, and colon. After intraperitoneal administration of NIV, no DNA damage was observed except in the colon [92].

5.6 Immunotoxicity

5.6.1 Effects on Immune Responses

As many scientists have indicated, NIV stimulates or suppresses the immune system depending on the dose as well as the presence of DON. Oral administration of 10 and 15 mg of NIV induced apoptosis in CD4(+) and CD8 (+) cells in thymus, Peyer's patch, and spleen in a dose-dependent manner [93, 94]. The effect of NIV on susceptibility against infectious diseases was shown by an in vivo infection experiment using BALB/c mice in which NIV at 6 mg/kg had no effect on survival rate against *Salmonella* infection [95]. However, in an in vitro experiment using RAW 264.7 cells, NIV inhibited the transcription activity and expression of inducible NO synthase (iNOS) by lipopolysaccharide (LPS) [96] (Sugiyama 2010).

5.6.2 Changes in Serum IgA Levels and IgA Nephropathy

Increasing IgA and induction of IgA nephropathy by NIV have been reported. These effects have been observed in mice but not in rats ([84], Table 100.6).

After C57BL/6 mice (ten males/group) were treated three times/week for 4 weeks by oral gavage with NIV (solvent: 5 % gum arabic water solution), the highest dose in that experiment (8.870 mg/kg bw) group showed a significant increase in plasma IgG but no changes in IgA [81], but 0.071 mg/kg bw/day and higher dose groups (0.355 mg/kg bw/day) showed a significant increase in plasma IgA [98]. When C3H/HeN, C3H/HeJ, and BALB/c mice (9–12 females/group) were treated for 4 or 8 weeks with feed containing 0, 6, or 12 mg/kg feed (corresponding to 0, 0.9, or 1.8 mg/kg bw/day, respectively) of purified NIV, the NIV-treated groups demonstrated an increase in IgA accumulation in glomeruli and increased serum IgA, particularly in the 12 mg/kg feed dose group at 8 weeks [99]. In a single oral administration study, Peyer's patches of BALB/c mice given NIV at 0 or 15 mg/kg bw showed a significant increase in all B cell subpopulations, particularly IgA + B cells, with the numbers of IgA + and IgM + B cells remaining higher than those of the control group [100] (#649).

Interestingly, an experiment using ovalbumin-TCR Tg (OVA-specific T cell receptor transgenic) mice (4 males/group) indicated that NIV significantly inhibited total IgE production and OVA-specific IgE, IgG1, and IgA production [101]. In F344 rats, NIV increased IgM level significantly but not IgG and IgA level even in the group given an oral dose of 6.9 mg/kg bw/day [84].

Table 100.6 Effect of NIV on IgA production

Study, strain, sex, age, group	Route of administration (solvent), period	Dose (mg/kg of diet)	Dose (mg/kg bw/day)	Critical effect	LOAEL (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Notes	References
Mouse, C57BL/6, 6 weeks	Forced oral dosage 4 weeks in 3 days/week		0, 0.014, 0.071, 0.355, 1.774, 8.870 mg/kg bw, 3 times/week	Increase in plasma IgG administration group of 8.870 mg/kg bw No changes in IgA		3.8[a]		[81]
Mouse, C57BL/6, 6 weeks	Forced oral dosage 4 weeks in 3 days/week		0, 0.071, 0.355 mg/kg bw, 3 times/week	Increase in the plasma of IgA	0.03[a]			[98]
Mouse, C3H/HeN, C3H/HeJ, BALB/c, 6–8 weeks	Diet, contam. 4 or 8 weeks	0, 6, 12	0, 0.9, 1.8[b]	Increase of serum IgA (with the increase) immunopathological change of the kidney similar to IgA-induced renal damage	0.9[b]		Used rice mold	[99]
Mouse, BALB/c, 5 weeks	Single, oral route (10 % DMSO)		0, 15	Increasing IgA cell in Peyer's patch cells, decreasing pan-T cell, pan-B cell in lymph node	15			[101]
Egg albumen (ovalbumin)-specific T cell receptor αβ-Tg mouse, BALB/c, 8–13 weeks,	Drinking water, 2 or 4 weeks	0, 6	0, 0.9[b]	Inhibition of anti-OVA IgE, IgG1 and IgA antibody, Inhibition of IL-4 production, induction of IL-2 production in spleen	0.9[b]			[101]
Rat, F344, 5 weeks (10 males and females/group)	Diet, contam. 90 days	0, 6.25, 25, 100	0, 0.4, 1.5, 6.9	IgM increase in the treated group 6.9 mg/kg bw/day No change in IgA and IgG		6.9		[84]
Pig, 51 days (6 males/group)	Diet, contam. 21 days	0, 2.5, 5		IgA in plasma had no significant difference compared with control				[86]

[a]Value corresponding to dose/day three times/week
[b]Estimated intake amount by corresponding value of EU Scientific Committee for Food

In pigs fed with purified NIV at 0, 2.5, or 5 mg/mg in feed for 21 days, no significant differences were observed in plasma IgA levels between the control and treatment groups [87].

6 Other Toxicities

NIV inhibits protein and DNA synthesis but not RNA synthesis [102]. NIV inhibited protein synthesis in rabbit reticulocytes with an IC_{50} value of 6 μg/mL [103]. Cytotoxicity of NIV, 4-acetyl NIV, and de-epoxy NIV on 3 T3 cell growth was IC_{50} 1.19 ± 0.06 mM (373 ± 20 ng/mL), 0.72 ± 0.04 mM (255 ± 13 ng/mL), and 64.2 ± 3.14 mM (19030 ± 930 ng/mL), respectively [104].

7 Outbreaks Associated with NIV

Outbreaks linked with *Fusarium*-contaminated cereal-based foods occurred in Japan and Korea during the 1940s–1960s [9]. The symptoms reported were nausea, diarrhea, and emesis. In China, more than 30 gastroenteritis outbreaks from 1961 to 1981 were associated with the consumption of "scabby," i.e., *Fusarium*-infected wheat, barley, or maize [8]. Trichothecenes were predicted to be the causative agent. An outbreak of trichothecenes occurred in the Kashmir Valley, India, during June to September 1987 in which 50,000 people suffered from gastrointestinal disorders [105, 106]. The cause of the outbreak was assumed to be the consumption of bread made from mold-damaged wheat, evidenced by the presence of molds such as *Fusarium* sp. and *Aspergillus* sp. and varying quantities of trichothecene mycotoxins. The concentration of DON, acetyl DON, NIV, and T-2 toxin in causative wheat was 0.34–8.4, 0.6–2.4, 0.03–0.1, and 0.55–4 mg/kg, respectively [105, 106].

8 Analytical Methods

Since NIV occurs as a co-contaminant with other trichothecene mycotoxins, it is often analyzed simultaneously with the co-contaminants rather than alone. Analytical methods developed so far include thin layer chromatography (TLC); capillary gas chromatography (GC) with electron-capture detection (ECD), flame ionization detection (FID), or mass spectrometric detection (GC/MS); high-performance liquid chromatography (HPLC) with ultraviolet (UV), fluorescence, or mass spectrometric detection; supercritical fluid chromatography (SFC); and time-of-flight mass spectrometry (LC/TOF-MS).

Usually, to analyze trichothecenes in foods and feeds, solvent extraction is essential. Aqueous methanol and acetonitrile are commonly used for extraction. For applying GC–MS, HPLC, and LC–MS/MS, a sample needs to be cleaned up with a charcoal-alumina-celite, florisil, silica gel, or solid-phase extraction column. For laboratory experiments, TL is very useful because of its low cost and simplicity.

However, for surveillance studies, trichothecene mycotoxins coexist with other trichothecenes, and a simultaneous analytical method for the determination of some trichothecenes and *Fusarium* toxins is considered to be more practical than a single method. A decade ago, GC was very popular to analyze some trichothecene mycotoxins in food, but for GC analysis, various derivatives are needed that are sometimes troublesome.

GC is typically used conventionally for simultaneous analysis for trichothecene mycotoxins and ZEN but requires trimethylsilyl derivatization before analysis. GC–FID produced good results in a validation of the EU Standards, Measurements and Testing Programme [107]. However, the GC method requires a derivatization procedure, which generally causes a loss of time and recovery.

On the other hand, LC using UV requires no derivatization procedure [108–111]. HPLC–UV (220 nm) for the GC–MS determination of DON and NIV has been developed in Japan.

Over several years, many LC–MS and LC–MS/MS methods were reported for the simultaneous analysis of trichothecene mycotoxins, including DON and NIV. These methods have been applied to the hygienic control and surveillance of mycotoxins. However, LC–MS requires the use of expensive internal standards such as isotopically substituted compounds. Meanwhile, the precision of LC–UV has been valued, although its sensitivity is lower than that of LC–MS.

A selective analytical method based on HPLC, combined with atmospheric pressure photoionization (APPI) mass spectrometry, has been developed for the simultaneous determination of NIV and DON. A liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI–MS) method based on time-of-flight MS (TOF/MS) with a real-time reference mass correction technique was also developed for the simultaneous determination of *Fusarium* mycotoxins (NIV, DON, fusarenon-X, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, HT-2 toxin, T-2 toxin, diacetoxyscirpenol, ZEN) and *Aspergillus* mycotoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2) in corn, wheat, cornflakes, and biscuits [112].

Sulyok et al. [113] reported the first validated method for the determination of 39 mycotoxins in wheat and maize by liquid chromatography with electrospray ionization–triple quadrupole mass spectrometry (LC/ESI–MS/MS) without the need for any cleanup. The 39 analytes included A and B trichothecenes (including deoxynivalenol-3-glucoside), ZEN and related derivatives, fumonisins, enniatins, ergot alkaloids, ochratoxins, aflatoxins, and moniliformin, and six trichothecene mycotoxins (NIV, DON, fusarenon-X, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and T-2 toxin).

Taken together, these analytical methods should be chosen for any purpose. For surveillance, GC–MS and LC–MS/MS are efficient because of their sensitivity, and for enforcement of regulation, HPLC would be useful.

9 Exposure Assessment of NIV and Regulation

Exposure to mycotoxins depends on the contamination level in different foods and on the intake of those foods, which is influenced by the dietary culture of each country.

Table 100.7 Analytical methods for determination of NIV

Extraction	Cleanup column	Derivatization	Determination	Toxins analyzed	Detection limit (NIV)	Commodities	References
CH_3CN—H_2O (85+15)	Charcoal-alumina-Ceite column	None	TLC $AlCl_3$ spray	DON, NIV, T-2, HT-2, DAS and two others	0.3–0.8 μg/g	Wheat, corn	[116]
CH_3CN—H_2O (3+1)	Florisil colum	TMCS	GC-ECD. GC-MS	DON, NIV	10 ng/g	Wheat, barley, corn and their product	[113, 114] 8.26–&$$ $;
MeOH+H_2O (7+1)	Silica gel column	TMCS, HFBA	GC-ECD. GC-MS	DON, NIV, T-2, HT-2, DAS and two others	20 ng/g (DON, NIV), 50–200 ng/g (HT-2, DAS)	Wheat, wheat flour, barley, corn flour, rye flour	[118, 119] 23.25–&$$ $;
CH_3CN—H_2O (85+15)	–(dilution only)	5	LC-MS/MS ESI	NIV, FX, T-2, DON, 3ADON, 15ADON	Not described	Rice medium	[120] Sagawa (2006)
CH_3CN—H_2O (84+16)	–(dilution only)	None	LC-MS/MS APCI	NIV, DON, D3Gl, FX, ADON, HT-2, T-2, ZON	5 ug/kg (NIV barley, malt)	Barley, malt	[121] Malachova (2010)
CH_3CN—H_2O—CH_3COOH (79+20+1)	–(dilution only)	None	LC-MS/MS ESI	NIV, DON, 3ADON and 36 others	20 ug/kg (NIV wheat)	Wheat, maize	[115] Sulyok (2006)
CH_3CN—H_2O (85+15)	MultiSep #227	None	LC-MS APPI	NIV, DON	0.2 ug/kg (NIV wheat)	Wheat	[111] Tanaka (2009)
CH_3CN—H_2O (85+15)	MultiSep #226	None	LC-TOFMS APCI	NIV, DON, 3ADON, and 13 others	2.4 ug/kg (NIV corn)	Corn, wheat, cornflasks, biscuits	[122] Tanaka (2006)

TLC Thin Layer Chromatography, *TMCS* trimethylsilylchlorosilane, *HFBA* Heptafluorobutyric anhydride, *HPLC* high-performance liquid chromatograpy, *GC-MS* gas-chromatograpy-mass spectrometry, *GC-ECD* gas chromatograpy with electron-capture detection, *LC-MS/MS* liquid chromatograpy-mass spectrometry, *LC-TOF/MS* liquid chromatography – time-of-Flight-mass spectrometry

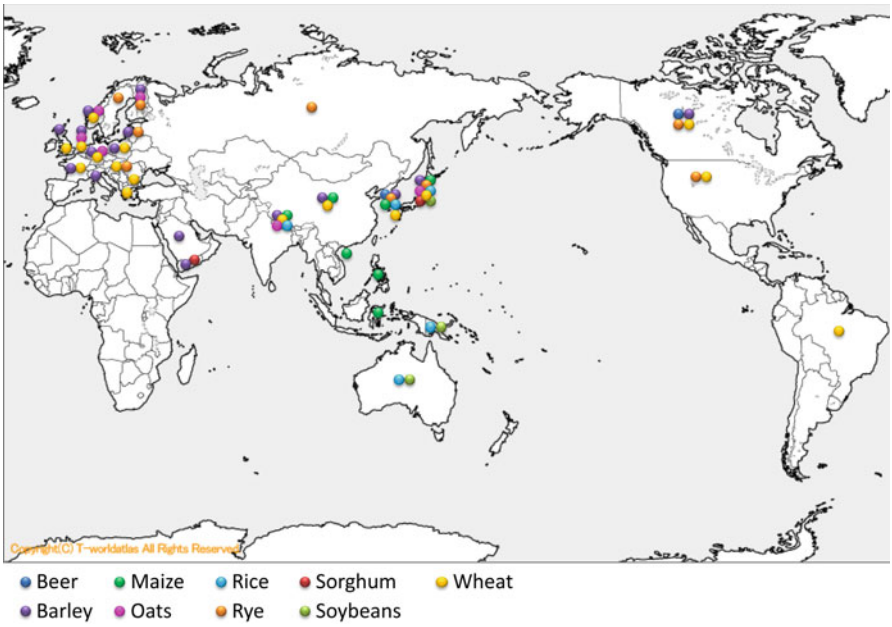


Fig. 100.5 Distribution of food contaminated with NIV (Modification cited by Ref. [120])

These data are based on established regulation levels of individuals or international institutes. For mycotoxins, it is ideal to correct the actual monitoring data over several years in raw and processed food. Especially, since exposure to trichothecenes and their toxicity are of concern in young children, it is important to assess the intake by age layers. FAO/WHO organized the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) and utilizes this information to assess the intake of mycotoxins (Table 100.7).

9.1 Occurrence of NIV in Food and Feed

Compared to the worldwide distribution of DON, NIV contamination is found only in limited areas. In Fig. 100.5, the commodities contaminated with NIV that have been reported are plotted [120]. Contamination of cereals (wheat, oats, barley, maize, rice, rye) has been frequently found in Far East Asia (China, Korea, Japan), Southeast Asia (the Philippines, Vietnam), Oceania (New Zealand, Australia) Europe (Germany, Poland, Norway, the Netherlands), and Eastern Europe (Lithuania). The contamination of soybeans (50 μg/kg of NIV) has also been reported in Australia [121].

From SCOOP data [11], food and food raw materials were shown to be contaminated by trichothecenes (DON, NIV, FX, T-2, and HT2 toxin, T-2 triol, diacetoxyscirpenol, neosolaniol, and verrucarol).

Table 100.8 NIV contamination in food in European Union member states

Commodities	% Positive	Mean (μg/kg)	Maximum (μg/kg)
Wheat	14	24	440
Maize	35	np	340
Barley	8	15	351
Oats	21	56	1,860
Rye	5	np	48

Adapted from Ref. [11]

Table 100.9 Occurrence of nivalenol in wheat in Eastern Asia (2002–2007)

Country	Commodities	Mean (μg/kg)	Maximum level (μg/kg)	References
Japan	Wheat	35	1,000	[122]
	Barley	172	3,000	
Korea	Maize	168	332	[123]
	Barley	390	2,038	[124]
	Rice (brown rice)	164	569	
China	Wheat	29	1,035	[125],
	Corn	18	1,400	

Table 100.8 shows NIV contamination in European countries. Among the cereals, mainly wheat, oats, wheat, barley, and rye are exposed to NIV. In Europe, the level and frequency of DON contamination are often higher than NIV contamination. Thus, it is generally believed that DON is more predominant than NIV and that their contamination levels shift in a parallel manner, i.e., when the DON level decreases, the NIV level also decreases. However, in the southern island of Japan (Kyushu), only the NIV-producing group strain exists (described in 1.3. mycology). Since commodities harvested from this area are contaminated with NIV, it has become a serious problem for human health.

Far East countries, including Japan, Korea, and China, are also suffering from NIV contamination of grain. In Japan, NIV concentration in barley was higher than that in wheat, while in Korea, barley was the most susceptible commodity contaminated with NIV. NIV contamination in rice has been reported in Korea but not in Japan. Since rice is the dietary staple in most Far East countries, even if the contamination level is low, it is of concern to human health (Table 100.9).

9.2 NIV Intake

The surveillance of NIV in food and estimated NIV intake were conducted in limited countries [22, 126]. Table 100.10 shows estimated NIV intake in the EU, UK, and Japan.

Table 100.10 Estimated NIV intake

Country	Age	Sex	NIV intake mean (95 % tile) ng/kg bw/day
Austria	Whole population		78 (274)
Denmark	Whole population		30 (72)
France	Adult		58 (99)
Finland	Adult		27 (np)
Norway	Adult	M	57 (110)
	Adult	F	50 (93)
Sweden	Adult		6 (13)
UK	Adult	M	25 (np)
	Adult	F	17 (np)
France	Children		94 (307)
Norway	1–6 years		113 (263)
UK	Infant		62 (np)
	1–4 years		64 (np)
	4–6 years		64 (np)
	7–10 years		50 (np)
	11–14 years		34 (np)
Japan	1–6 years		10 (330)
	7–14 years		10 (230)
	15–19 years		10 (180)
	>20 years		>10 (110)

Adapted from Refs. [11, 122]
np not provided

As shown in Table 100.10, viewing the entire population, the highest mean and 95th percentile of estimated NIV intake was shown in Austria. Among the exposed adults, in France, the estimated intake of NIV was highest compared to adults of other countries. However, the intake by children was higher than by adults. In young children, mean intake in the UK was 62–64 ng/kg bw/day, and these values were higher than in Japanese children.

9.3 International Evaluation

The FAO/WHO Joint Expert committee of Food Additives has not evaluated NIV contamination levels. The IARC has evaluated carcinogenesis of the toxins produced from *F. graminearum*, *F. culmorum*, and *F. crookwellense*, such as ZEN, DON, and acetyl NIV [127]. Their conclusion was that metabolites of these three *Fusarium* species should be placed in group 3, i.e., not classified as carcinogenic for humans.

The Scientific Committee for Food (EC) reported values for DON in 1999, for NIV in 2000, and for T-2 and HT-2 toxins in 2002[128]. According to their opinion,

temporary tolerable daily intake (t-TDI) of NIV was estimated to be 0.7 μg/kg based on NOAEL and 0.7 mg/kg temporary bw based on the results of 1- and 2-year repeated dose studies reported by Ref. [77, 88].

In 2010, the FSC of Japan evaluated DON and NIV and concluded that the provisional maximum tolerable daily intake (PMTDI) of NIV was 0.4 μg/kg bw/day based on LOAEL (0.4 mg/kg bw/day) of subacute repeated dose toxicity studies using purified NIV [86]. As the PMTDI of DON was adopted to be 1.0 μg/kg bw, it had the same value as JECFA and EFSA.

9.4 Regulation

To date, no country has established a standard for NIV yet. However, regarding the risk assessment of NIV, Japan and EU lead the world. These countries have serious problems related to the management of NIV-producing fungi. The establishment of a regulation level for NIV is one of the effective means to prevent contamination of NIV in cereal grain.

10 Conclusion

Because NIV is a minor mycotoxin in the world, information for risk assessment is very poor. Of course, European countries and Japan have recognized the threat posed by NIV to human health and have started to evaluate this risk. In the risk assessment of NIV, it is recommended to take into account the effect of co-contamination with other trichothecene mycotoxins, namely, DON. As further studies, combination toxicology studies of NIV and DON are needed.

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